1959 REPORT
of the
SCIENTIFIC DIRECTOR

CLARENCE COOK LITTLE, Sc.D.

TOBACCO INDUSTRY RESEARCH COMMITTEE

Organization and Policy

The Tobacco Industry Research Committee is the sponsoring agency of a research program into questions of tobacco use and health. It was organized in early 1954 by representatives of tobacco manufacturers, growers and warehousemen.

Soon after, the T.I.R.C. invited doctors and scientists well known for their work in cancer and other diseases to serve on a Scientific Advisory Board. This Board consists of 10 scientists who maintain their respective institutional affiliations.

The Scientific Advisory Board has full responsibility for research policy and programming. As a Board, it does not engage in research for the T.I.R.C. and the T.I.R.C. itself does not operate any research facility.

Grants-in-aid for research are made by the Board to independent scientists who are assured complete scientific freedom in conducting their research. They alone are responsible for reporting or publishing their findings in the accepted scientific manner — through medical and scientific journals and societies.

From the outset, the Tobacco Industry Research Committee has made clear that the object of its research program is to encourage scientific study for facts about tobacco use and human health. Its position remains that research will help provide the knowledge about lung cancer and heart disease for a full evaluation of all factors being studied in connection with these diseases.

Grants recommended by the Scientific Advisory Board through 1958 have been made to 90 scientists in 61 hospitals, universities and research institutions from funds that, so far, total $3,700,000 appropriated by the T.I.R.C.

A list of research projects supported by the T.I.R.C. is included in this Report. Also included are abstracts of 38 research papers acknowledging support by the T.I.R.C. that have appeared in scientific journals since the 1958 Report and through July 1959. A total of 91 papers acknowledging T.I.R.C. support have now been abstracted in the Annual Reports.

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TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East 42nd Street, New York 17, N. Y.

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to the Tobacco Industry Research Committee

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Preface

The effort to obtain new knowledge of the origin and nature of the so-called constitutional or chronic diseases is wide-spread.

What are the problems? What has been accomplished and what remains to be sought for, discovered and analyzed?

Very evidently no complete answers to these questions can yet be given. One may, however, comment on certain evidence of progress and discuss trends and prospects that seem to be worthy of attention.

It is hoped that this Report will serve to maintain and stimulate interest in, and support of, the vast amount of research that still needs to be done before our knowledge and techniques can control or prevent these diseases.

As Scientific Director, I should like to acknowledge the devoted and unselfish efforts of my associates on the Scientific Advisory Board and also the cooperation and creative contributions of our grantees and scientific consultants. I should also like to emphasize again the vital part in our whole effort played by all members of the Tobacco Industry Research Committee in providing complete freedom of planning and action to those who are responsible for its scientific research program.

C. C. Little
Scientific Director

Current Status of Tobacco-Health Studies

The past year has produced an interesting and provocative situation arising from the activities of different research workers independently investigating the possible role of smoking as one of many factors being studied in human health problems.

What happened in 1959 was the appearance of many more research findings and comments clearly showing the uncertainties and divergence of opinion among doctors and scientists in regard to tobacco and health. Diversity of research problems and speculation about them have existed since 1954, but in the past year they became more marked in articles and discussions referring to smoking.

These things, of course, often characterize any phase of a complex research problem and seem to arise especially at the stage when the urge to solve any problem exceeds the knowledge necessary to enable us to do so. This is not a cause for alarm or fundamental disturbance of any kind. It is the reflection of natural evolution of our knowledge of the problem and its effect upon the thinking of the people engaged in efforts to find its solution.

THEORIES ABOUT TOBACCO

As an example of such evolution, we may cite several theories that have been postulated in attempts to explain the limited data that appear to indicate the supposedly harmful effects of smoking, especially with respect to cancer of the lung. On present evidence, any one or some combination of these concepts, or of others yet to be offered, may be true, or none of them may be. Among these theories are:

Theory 1. ***There are those who suggest there may be carcinogenic substances in tobacco smoke that might cause human lung cancer by direct action.***

Practically the only support for this theory is the reaction of the skin of some laboratory animals, notably certain mice, to the application of concentrated condensates of tobacco smoke produced by one mechanical device or another.

However, no substance has been found in tobacco smoke in quantities sufficient to account for even the mouse skin reactions that have been reported. Also, there are many reports of mouse skin-painting experiments that did not result in tumors.

Repeated experiments in this country and abroad in which laboratory animals were induced to inhale tobacco smoke *have failed to produce a single lung cancer of the type that is prevalent in man.*

It is generally accepted that lung tissue of mice is more comparable to lung tissue of man, and that smoke directly applied to the target tissue by inhalation is more like the actual process of human smoking than is the application of heavy doses of smoke condensates to mouse skin.

There has been growing recognition of the importance of the so-called negative evidence obtained from inhalation experiments. The work of Passey, described in the report of the British Empire Cancer Campaign, issued in the summer of 1959, made this clear and gave actual instances of the experimental work done. Scientists and the public will recognize that there is more direct and applicable evidence in this inhalation research than in the mouse skin-painting type of work.

Theory 2. ***Some persons hold that smoke may act as an irritant contributing to the increased probability of malignant changes that primarily result from other intracellular factors.***

This theory, essentially based on the assumption that tobacco may have a contributory effect, has not been studied to any great degree and therefore needs further exploration and testing. The various methods of assaying the effects of tobacco smoke must be investigated further to find out whether the responses of the target tissue are specific for tobacco smoke, or whether they represent a general reaction that may be started by a number of different internal and/or external agents.

Theory 3. ***Some suggest that excessive smoking produces a "general debility" that increases the probability and hastens the appearance of a whole array of pathogenic phenomena.***

The term "general debility" is a loose one, and supporters of this idea are unable to define it. Debility may be caused in different people at different times by different circumstances and different agents. It may describe different types and degrees of internal disturbance and unbalance. Also, "excessive smoking" and "excess" in any other human habit are further variables that make this theory difficult to prove or to disprove, or even to define in a concrete and analyzable form.

What may be "excess" for one person may not, in any appreciable way, affect the physiology of another. If tobacco is to be classified in the category of agents that produce "general debility," it will join a whole host of other agents and influences that presumably can do the same. To separate the supposed effects of tobacco from effects of other things will

be a most difficult task and will require a great deal of planning and accumulation of fundamental research to find out what really are the best methods of assay.

This particular concept seems to have originated in an effort to interpret the reported statistical association between tobacco use and various ailments for which there was no satisfactory evidence of direct causation.

Theory 4. ***The degree of use of tobacco may be diagnostic of types of persons who have different health risks because of their innate nature, or their rate of living and reactions to the various physical, mental and emotional challenges and stresses of life.***

The data concerning this theory have been accumulating rather rapidly and from a number of different sources. One is the continuation of studies of human twins, both identical and non-identical, that indicate a constitutional factor that may influence smoking habits. Recently four Swedish scientists published a study that in many ways paralleled one Sir Ronald A. Fisher reported last year. Their study came to the same conclusions that he did, namely, that identical twins more often had the same smoking habits than did non-identical twins, even though the degree to which both types of twins encountered a similar environment was essentially the same.

Other data have come from several types of psychological tests that are being given to "captive" populations of people to find out the mental and emotional traits of smokers and compare them with those of non-smokers. A recent paper reported on certain significant mental and emotional differences between the two categories. Although its author did not interpret it as conclusive that the type of person is an important factor in the situation, the fact remains that a difference was demonstrated. This difference will have to be explained and evaluated along with other associations of a statistical and semi-statistical nature.

This last theory really indicates that "excessive" smoking is symptomatic of a certain type or types of person and is *not* a prime factor in creating or establishing the types of persons who are bad health risks. Therefore, it is not so appealing to those who are pledged to substantiating a causal relationship.

We find that we now have at least these four theories, the absolute and relative importance of which is uncertain or unknown. We do not *know,* and *that* is the important thing to remember. Further research is needed. The present situation indicates this unequivocally, and it proves the wisdom of the attitude of scientific conservatism.

OBSERVATIONS ON RESEARCH

In the 1958 Report it was pointed out that the proponents of the theory of a tobacco link to lung cancer had rested their claims on a "tripod" of data — statistics, pathology, and animal experimentation. In the past several years each leg of the "tripod" has been subject to more and more analysis by independent investigators who have pointed out the weaknesses, deficiencies and even contradictions in the results.

Statistics

During 1959 additional statistical material reporting an association between smoking and certain causes of death appeared to be much the same as earlier data. There was considerable criticism of the conclusions drawn from this type of statistical work when the original data appeared and this criticism has continued.

Two individuals in particular have for some years been closely analyzing and commenting on the statistical work and the over-interpretation of it that has been so well publicized. These are Dr. Joseph Berkson, head of the Section of Biometry and Medical Statistics at the Mayo Clinic, Rochester, Minn., and Sir Ronald Fisher, a former Arthur Balfour professor of genetics at the University of Cambridge, England.

Dr. Berkson, a member of the American Cancer Society committee that sponsored a numerically large statistical study of smoking and death rates, has published several papers relating to statistical investigations of tobacco and lung cancer. He has said that it was premature to claim on the basis of statistical findings that smoking causes cancer of the lung.

Cancer Biologic Problem, Not Statistical

Cancer, according to Dr. Berkson, is basically a biologic, not a statistical, problem, and the statistical conclusions would have to be corroborated fully by experimental and direct observational studies before they could be considered to be scientifically established. There is, he said, virtually no substantial clinical, pathologic or other direct evidence that smoking is the cause of lung cancer.

Sir Ronald, in a recent pamphlet containing his various published papers and lectures dealing with smoking, comments pointedly on the statistical work of two British investigators whose data indicated that inhalation of cigarette smoke actually seemed to *diminish* the chance of lung cancer in the population studied.

Sir Ronald, in commenting on this, has said: "There is nothing to stop those who greatly desire it from believing that lung cancer is caused by smoking cigarettes. They should also believe that inhaling cigarette smoke is a protection. To believe either is, however, to run the risk of

failing to recognize and, therefore, failing to prevent other and more genuine causes."

Statistical Data 'Logically Incompetent'

Other pertinent remarks on the statistical studies have come from Dr. Horace W. Norton, professor of statistical design and analysis at the University of Illinois College of Agriculture, who described the present data on smoking and lung cancer as "logically incompetent."

He further wrote in a recent published communication: "I make a plea for integrity on the part of those affirming that smoking causes lung cancer. Let their various papers and public statements include (1) a frank acknowledgement that any such affirmative conclusion is a mere *opinion* and (2) a familiar standard of comparison, choosing something over which we have, or ought to have, some voluntary control, such as the death rate associated with the use of the automobile."

Additional significant comments have been made by Dr. J. Yerushalmy, School of Public Health, University of California at Berkeley, and Dr. Carroll E. Palmer, U.S. Public Health Service's Tuberculosis Program, in a discussion of the statistical work in a recent article on the methodology of investigations of etiologic factors in chronic diseases.

The large number of smokers who do not have lung cancer "testify to the insufficiency of smoking as a cause of the disease," they wrote, noting that "the existence of lung cancer patients who have never smoked clearly indicates that smoking is not a necessary cause."

A great deal of research needs to be done to find out where the truth lies—if, in fact, it lies in any of the existing conjectures, which it very well may not. There is growing support for the point of view that the statistical association claimed by various studies has an explanation or explanations that may still not be apparent from our present knowledge.

Pathology

The second leg of the tripod—pathology—has rested on very limited material. On the basis of a relatively few cases, it was claimed that the lungs of smokers contained characteristic lesions, or spots of damaged tissue, that were considered to be precancerous conditions. In fact, these spots were called "carcinoma *in situ*" (localized cancer)—in itself a confused and contradictory term.

Very early, the Scientific Advisory Board to the Tobacco Industry Research Committee sponsored a long-time study of the pathology of human lungs by 12 leading pathologists in different parts of the country. All these pathologists found various lesions frequently in lungs of non-smokers as well

as in smokers, in persons of both sexes and of all ages and of different places of residence. They did not believe that these lesions were indicative and diagnostic of precancerous conditions.

Confirmation of the need for caution in interpreting such studies came this year from Cunningham and Winstanley in England. In pathologic studies of human lung tissues, they were unable to confirm findings of "carcinoma *in situ*" or of an association of certain other changes with the smoking history of the patients.

Animal Experimentation

The third leg of the tripod, animal experimentation, has relied mostly on continued studies in a few laboratories. It has been reported that repeated painting of condensates of smoke on the skin of certain strains of mice was followed in some of these animals by overgrowth of the skin and, sometime, by cancer of the skin. From such data, it was argued by a few that there might be substances carcinogenic to people in the smoke condensates and, therefore, by inference, in the smoke of a cigarette as consumed by a smoker. On the basis of these animal painting experiments, the theory was propounded to the effect that if the solids in cigarettes smoke were reduced, the amount of skin cancer on mice would be reduced, and the chance of human lung cancer would be similarly reduced. However, an attempt at quantitative extrapolation from mice to man would exceed scientific limits.

The continuing reports of failure to induce lung cancer in animals with direct inhalation of cigarette smoke itself are considered by many to be more significant than skin painting, because smoke itself is utilized and the tissue challenged is lung, not skin, tissue. In any event, these results have called for a re-evaluation of the smoke-condensate, skin-painting arguments on which, up to now, the whole "third leg" of animal experimentation has rested.

Question of Air Pollution

Experimental work has indicated that adverse effects on health may result from the presence in man's environment of "smog" or combustion products of gasoline, oil, gas, coal, or other air pollutants. Of course, the existence of these factors does not mean scientists should disregard tobacco and other factors—either internal or external—in their investigations. However, the fact that there are other suspects in lung cancer, cardiovascular disease, and other ailments should conceivably affect and modify the over-interpretation with regard to tobacco.

A recent study in South Africa suggests air pollution may be involved in the causation of lung cancer. A report by Dr. Geoffrey Dean, published in the *British Medical Journal*, noted that white male South Africans have

long been the heaviest cigarette smokers in the world and yet they have a relatively low lung cancer mortality rate.

His study, based on 1947-56 male lung cancer deaths in the Union of South Africa by age, country of birth and place of residence, showed that British immigrants to South Africa had much higher lung cancer mortality rates than Union-born men or immigrants from other countries.

Dr. Dean said: "The relatively low incidence of lung cancer generally among the heavy-smoking South African men, the higher and rapidly increasing incidence in the growing cities, and the high incidence in the younger age group of immigrants from Britain found in the present study, suggest that the air pollution which occurs in modern industrial life — smoke, smog, traffic fumes, etc.—may be a major factor responsible for the alarming increase of lung cancer in South Africa and Britain, and presumably elsewhere."

Dr. Dean's findings are similar to those reported three years ago by Dr. David F. Eastcott, assistant director of the National Health Institute of New Zealand. Dr. Eastcott also found that immigrants from the United Kingdom had a higher incidence of lung cancer than native-born persons of the same stock and that differences in tobacco smoking did not seem to be involved.

There is, therefore, an obvious and pressing need for investigating the possible role of such air pollutants in lung cancer and other diseases. The existence of these and other suspected factors justifies the attitude of balanced scepticism toward claims that the problem is solved and the insistence on further research adopted by the Tobacco Industry Research Committee and others.

The T.I.R.C. Research Program

BASIC PRINCIPLES

Certain basic principles influence the long-range planning of the Scientific Advisory Board, which is responsible for the recommendations for distribution of research funds from the T.I.R.C.

These principles are broad and presumably admissible by all those interested in advancing knowledge of the cause and prevention of those constitutional or chronic diseases attributed by some, at least in part, to tobacco use.

Lung cancer and cardiovascular disease are at present emphasized in the T.I.R.C. program, but other diseases and research problems also are included.

Cancer and cardiovascular disease are due to changes in the cells and tissues of living people. Both commonly occur after a person has lived for a number of years, and sometimes after a great number. There are wide and unexplained individual variations and susceptibilities in this respect.

Cancer is usually microscopically local in origin, i.e., in a single cell. Cardiovascular disease commonly originates as a result of changes in tissue or system of tissues, i.e., thousands of millions of cells are simultaneously or coordinately affected.

The origin of each category is extremely complex and is undoubtedly due to many independent and interacting causes. It must be recognized and admitted that our knowledge in these fields is markedly imperfect and deficient. The multiple causes and influences may operate in different degrees, in different order, and with different relative importance in different human individuals.

These general principles should constantly be borne in mind by those supporting research in either field, by those doing and interpreting the research, and by those informing the public about prevention, causation, treatment or cure.

Because of the number and variety of these principles, research programs should be diversified in order to advance our total knowledge with the greatest possible speed and balance. Any *one* phase of research or research approach is, by the very nature of the diseases, limited in its potential value and in its application to the total problem.

FACTORS IN CANCER-CARDIOVASCULAR DISEASE

Broadly speaking, cancer and cardiovascular diseases involve at least the following fields:

1. *Heredity*—what effect does it have on the biochemical and genetic nature of the individual? Some individuals in the same environment develop these diseases while others do not.
2. *Infection*—how much do bacteria and/or viruses, either present or previously experienced, influence cell or tissue changes? To what extent do they increase the risk of later disease?
3. *Nutrition*—how much do the various nutritive materials taken, absorbed, stored or excreted by the individual affect cell or tissue changes? Cholesterol is one substance now under extensive investigation, but vitamin deficiencies and other unbalances may be important.
4. *Hormones*—how much do the products of the various glands of internal secretion, transmitted by the body fluids, affect cells or tissues either by amount and rate of secretion or by unbalance? It is known that they have an important role in breast cancer and adrenal cancer, and that men have four to six times as much lung cancer as women.

5. *Nervous strain or tension*—how much do these factors influence the activity and function of cells and tissues of various systems of the body other than the nervous system itself? Ulcer is recognized as a disease in which stress is important. Stress is apparently also involved in cardiovascular disease.

6. *Environment*—how much do physical or chemical components of the environment, introduced as foreign non-living agents, affect the cells or tissues? Air pollutants, irradiation, humidity, temperature, tobacco, occupational exposure and possibly other factors are involved in this question.

Studies of these fields are important to obtain a better and, eventually, a complete understanding of cancer causation, and all, or a majority of them, are similarly to be considered in the causation of cardiovascular disease. The Tobacco Industry Research Committee has supported and initiated research in these fields and will expand its activity as opportunity arises or can be created.

DEVELOPING LINES OF RESEARCH

In the past, certain lines of research have been mentioned that seem to give promise of contributing knowledge essential to the understanding, analysis, and eventual solution of some of the major problems related to the cause and development of the constitutional or chronic diseases. These lines of research include:

1. Tissue Culture

The training of fellows and the development of techniques in *in vitro* culture of human lung tissue have proceeded satisfactorily at Johns Hopkins University, the University of Nebraska, the University of Texas, and the Albert Einstein School of Medicine.

The discovery by a researcher of at least one entirely synthetic culture medium for mammalian tissue is a welcome and long-awaited key that should open the door to a whole new order of qualitative and quantitative analysis of the phenomena of growth in both normal and neoplastic tissues.

2. Bioassay

Distinct advances have been made in initiating large-scale research designed to provide the careful controls essential to successful, quantitative standardization of such widely used methods of assay as skin painting or injection with various agents.

Assay by rate of growth of, or reactions to, transplanted normal and neoplastic cells and tissues of known genetic nature in genetically controlled hosts is also well under way.

The effects of known challenging agents on the survival of pure clones of *Paramecium* are also being investigated in the hope that such assay may

prove to be a rapid, effective, and convenient adjunct to other types of assay.

Comparative results of several different types of assay, when the same agents are used in each, may well contribute significantly to our ability to understand the total process of assay more completely.

3. *Influences of Concurrent or Previous Infection*

The question whether neoplasia may occur at or near areas of infection has been raised by many investigators over a considerable period of years.

There are at least two broad fields of interest involved that are being investigated both experimentally and clinically at present.

A. Do areas of tissue, damaged or altered by previous infection, become subject to malfunction of intercellular or inter-tissue exchange? Could this in turn lead to chemical unbalance resulting in intracellular change or mutation from a normal to a neoplastic type in adjacent or surrounding tissue?

B. Do invasive agents, such as non-pathogenic or latent or partially inactivated viruses, exist in non-neoplastic cells and, if so, what capacity do they possess for transformation to an active state within the cell that leads to its uncontrolled division?

4. *Psychosomatic Influences*

There has been a continuing growth of interest in the psychosomatic aspects of smoking. The number of discussions and publications in this field has increased markedly, as have plans for, and inception of, new research. This indicates that the output of research and its importance in obtaining a well-rounded picture of the smoking habit will grow.

There are and will be many difficulties in the development of this field on the basis of direct, observational, and experimental methods.

Some of these difficulties will be inherent in the nature of the human material used, such as the establishment and maintenance of adequate follow-up techniques so necessary to longitudinal studies.

Others will involve careful evaluation of various methods of recording and assaying various psychological or psychosomatic characteristics and processes.

Even now it is possible to reach certain general conclusions on the relation of behavioral traits to the incidence, development, and course of the constitutional or chronic diseases. It should be recalled that it is chiefly in that group of diseases that certain statisticians have reported association between "excessive" cigarette smoking and pathogenesis.

5. *Genetic Influences*

The genetic influence is often misunderstood and misinterpreted. For example, it seems to be a common error to expect that the genetic (innate) element or factor in this complex problem will be a single, clearcut, Men-

delian gene for "smoking" or "non-smoking"; for "lung cancer" or for "no lung cancer"; for "cardiovascular disease" or for "no cardiovascular disease."

The observed behavior traits of individuals in relation to these diseases preclude the existence of a simple uni-genic relation as being of prime importance.

Statements of this kind regrettably have led a number of individuals to discount *all* genetic influence and lose interest in the evidence of complex but nonetheless real genetic influences at work. This is, of course, a tragic error in analytical technique.

Studies of twins, as well as of various psychological and emotional traits, show that there can be strong genetic predeliction to certain abilities, skills, and reactions. These genetic influences often differ in strength and in tenacity of recurrence and of expression—even when environmental factors are held constant. Individuals may still differ in response because of their innate characteristics.

To complicate matters further, the environment, and the challenges that it presents to implement or to obstruct the development and expression of innate characteristics, usually change radically in various ways, tempos, intensities, persistence, and degree.

Some genetic responses occur with clarity and definiteness when the environment presents a clear and fixed challenge. This situation is, however, the marked exception. Much more often there will be counterplay and interplay of the innate and of the environmental influences, resulting in a changing, living competition.

At one age and under one set of circumstances the innate influences may be in control, while at another age and under different circumstances, environmental influences may determine what occurs.

There is no doubt that different individuals have different inherited tendencies to develop at certain rates in various tissues and organs. There are hereditary tendencies for different organs and tissues to age at different rates. All of this complex internal balance and counter balance affects susceptibility to pathogenic changes and disease.

6. *Strain or Tension*

The stress or tension factor is beginning to assume more importance. This factor appears at times to be associated with the smoking habit. Also, it is well recognized as a predisposing and/or contributory factor to certain diseases.

In evaluation of the strain-tension factor, it is well to remember that sensitivity to recognition of strain varies greatly in individuals. So do retention of the effects of strain and of neurophysiological reaction to it.

The genetic makeup of the individual and the type and strength of environmental stimuli are again the factors that determine behavior reactions to strain, tension, fatigue, frustration, competition, and a whole host of the component problems of modern life.

The rate and amount of cigarette smoking follow very closely the exposure of certain individuals and populations to situations involving increasing or decreasing stress and strain. For example, in World War II there was a marked rise in per capita cigarette consumption both among civilians and military personnel.

There is evidence suggesting that the smoking of an individual is determined by his inner reactions as shaped by extremely complex and varied genetic and environmental factors. In other words, the smoking habit may be a *reflection* of traits rather than a *determiner* of them. This subject deserves further exploration.

SUMMARY AND CONCLUSIONS

It will be readily seen from the discussion of various aspects of tobacco and health that the subject is broad and the problems complex.

The important point is that an accumulation of amounts of circumstantial or inferential data is not a substitute for experimental and clinical evidence based on direct observation.

On December 12, the *Journal of the American Medical Association,* commenting editorially on "Smoking and Lung Cancer," said: "Neither the proponents nor the opponents of the smoking theory have sufficient evidence to warrant the assumption of an all-or-none authoritative position."

There are many persons, both intelligent laymen and scientists, who will agree with that statement. They will not accept a simple cause and effect relationship in cancer and cardiovascular disease unless such a relationship can be proved by something more than disputed statistics, transferred interpretation from animal work, or limited autopsy findings. Neither will they reject a possible role for tobacco along with other environmental exposures, until evidence permits a true evaluation.

Beliefs Upheld By Past Experiences

The experiences of the past six years amply justify and support these beliefs held by the Tobacco Industry Research Committee and others:

1. Any role of cigarette smoking in lung cancer and certain other diseases has not been proved *as causative.*
2. If tobacco has any role, it is uncertain, unidentified, and unanalyzed.
3. Much more research is needed to help clarify and define the

significant problems, and to determine the best way to find the answers to them.

4. *All* evidence, including that which demonstrates the gaps and uncertainties and contradictions in our knowledge, should be presented to the public honestly and fully. The individual can form his own considered opinion only *on the basis of complete information.*

A Balanced Attitude

The Tobacco Industry Research Committee believes in the creation and maintenance of a balanced attitude toward the tobacco and health situation. It does not believe that the situation has been solved, that the problem has been taken care of, and that the answers are known. We do know that the problems in cancer and cardiovascular diseases are tremendously complicated and the chance of finding a simple answer is small or non-existent.

Let us be perfectly sure of what we call established causal factors, and let us be honest in our evaluation of what we advocate—of the soundness and value of evidence—before we attempt to convince the public of any proven guilt or lack of guilt in any widespread human usage or custom, whether tobacco use or something else.

While this attitude has been criticized by a few as being obstructive, it is actually, from the long-time point of view and for the good of science and humanity, far from being so. It is much better to proceed accurately and slowly to results that will last, than it is to proceed sensationally and rapidly to tentative conclusions and part-truths that may later have to be abandoned.

The Tobacco Industry Research Committee will insist on continuation of conservative, objective, and painstaking research on which to establish and extend human knowledge in fighting the constitutional diseases. These diseases are and will be man's great peacetime survival problems.

Results of Research

The progress of the research program has been reflected in a constantly growing number of published papers that are abstracted individually and listed elsewhere in this Report. Nevertheless, in order to provide some perspective of the plan and scope of the program and of the present state of knowledge, it seems pertinent also to summarize the findings and techniques in several important fields of investigation. At some points the summaries may touch upon work whose results have not yet been published in full. Naturally, not all the work under way or completed is discussed here.

These summaries are: I. Smoke Inhalation Studies; II. Lung Pathology; III. Carcinogenicity and Bioassay; IV. Tissue Culture; V. Cardiovascular Research; VI. Psycho-physiological Studies, and VII. Miscellaneous.

I. Smoke Inhalation Studies

The T.I.R.C. has sponsored a number of studies in which experimental animals were induced to inhale cigarette smoke. Some of these are far enough along to indicate certain findings.

In one study scientists exposed mice of two strains to cigarette smoke inhalation in a machine which controls the puffing conditions to simulate closely the manner of human smoking, for periods approaching the entire normal lifespan of the animals after weaning. No lung cancers of the type prevalent in humans were produced. Lung adenomas of the type that occur naturally in these mice occurred no more frequently than in unexposed mice.

Another researcher studied the effect of smoke inhalation by CF_1 mice which were also painted on the skin with the potent carcinogen, methylcholanthrene. No synergistic effect was observed.

In a third study two scientists set up the necessary machinery for introducing fresh cigarette smoke directly into the lungs of dogs through artificial openings in their tracheas. The smoke was produced under carefully controlled conditions of puffing volume, duration and frequency in order to assure that the composition of the smoke would resemble closely that of smoke normally inhaled by humans. The study was continued for more than three years and individual dogs received smoke of 10-20 cigarettes daily for many months. No lung cancers developed.

In another laboratory scientists have recently undertaken to subject mice to cigarette smoke inhalation for a somewhat different purpose. Having found that the susceptibility of mice to lung adenomas of the type that can be induced by feeding them urethane depends upon the supply of niacin, they

wish to determine whether any ingredient of tobacco smoke can influence the effective supply of this vitamin either directly or indirectly.

Another investigator has done the most extensive study that has been sponsored by T.I.R.C. on effects of cigarette smoke inhalation by mice. Groups of female CF_1 mice were exposed five times a week to the smoke from four to six cigarettes over a period of nearly two years. No invasive carcinomas of the lung were found, although some mice developed a bronchitis. The occurrence of adenomas in these exposed animals was no greater than that normal to the strain without smoke exposure.

II. Lung Pathology

Work in the field of lung pathology has been of two types: studies of human lung tissues acquired at autopsy, and studies of animal lungs following controlled smoke exposure.

STUDIES OF HUMAN LUNG TISSUES

Early in 1955 twelve pathologists located in hospitals in various parts of the country from coast to coast undertook a coordinated study to determine what kinds of abnormalities occur in the lungs of human subjects that come to autopsy. The incidence of such abnormalities was to be determined and the relation of the incidence to age, sex, occupation, residence, environment, and cause of death was to be determined as far as possible. Six classifications of cellular conditions were adopted and about 2000 lungs were collected and examined over a period of two and a half years. It was found that data could not easily be pooled because of differences in the classification of tissue sections by different observers. These differences were revealed by circulating two slide collections under code for independent classification by several pathologists.

Preliminary conclusions from this study were:

1. There was no age difference in the prevalence of changes, classed as "hyperplasia or metaplasia," at any age above 25 years. Less than 30 percent of all cases studied, including both sexes and all ages, had mucosae classed as "normal" in all the sections that were examined.
2. Males showed changes departing from "normal" more often than females, but both metaplasia and hyperplasia did occur frequently in females.
3. Cigarette smokers in this study tended to have fewer "normal" sections than non-smokers.
4. By a rough division of occupations into "hazardous" (those which may have a high death rate from lung cancer), those in farming, and others, no consistent excess of metaplasia or hyperplasia appeared in any of these three groups.

5. Study of the occurrence of metaplasia or hyperplasia by the size of the largest city in which the cases ever resided revealed no significant differences.

6. Metaplasia was present more frequently in those dying from lung cancer than in those who died from other cancers or from all other causes.

7. "Carcinoma-*in-situ*" (a classification that has been used by some pathologists to describe a condition that looks microscopically like cancer but has not shown invasive growth) was rarely diagnosed by any of these 12 pathologists, and then usually only when invasive cancer was also present.

One pathologist is continuing a part of this study by making a more minute examination of his collected lungs by serial section. Conditions of the lungs will be compared in relation to residence environment.

The relation of metaplasia or hyperplasia to cancer is unclear because of the differing concepts as to how cancer arises in tissue. Opinions differ widely as to whether such conditions are to be considered as "precancerous" in any sense.

STUDIES OF ANIMAL LUNGS

From studies of the cellular condition of mouse lungs following exposure of the mice to cigarette smoke inhalation under relatively well-controlled conditions, several important findings have resulted:

1. Some of the mice exposed to smoke inhalation develop bronchitis—a chronic inflammation of the bronchial tubes. Others show no change from normal whatever.

2. The incidence of bronchitis in the mice varies greatly from one group to another even though the animals are from inbred stocks presumed to be identical and have received the same diets, care, and treatment so far as known.

3. The number of cases of bronchitis in any group or the severity of the inflammation seems to bear no relation either to the intensity of the smoke dosage or to the length of time that the exposure was continued.

4. The bronchi of mice with bronchitis show various degrees of hyperplasia and metaplasia very similar to that often found in human lungs.

5. Continuation of the smoke inhalation during the entire life-span even of the bronchitic mice has not produced cancer.

6. When smoke exposure is discontinued in the case of bronchitic mice, the abnormalities (hyperplasias and metaplasias) tend to disappear.

These observations cast doubt on the interpretations that have been applied by some pathologists to similar abnormalities in human lungs as "precancerous." Moreover, the manner in which only certain mice develop

bronchitis when exposed to smoke points strongly to a *difference in susceptibility* in the mice themselves, of a nature not yet disclosed. A possible hypothesis, which is being pursued, is that some of the animals may have a latent virus infection that becomes active. The explanation of this phenomenon may produce some valuable leads for human studies since humans also appear to differ greatly in their responses to smoke inhalation. However, these experiments so far reveal no relation between bronchitis and cancer of the lung in the mice.

Conference on Chronic Lung Diseases

On September 11, 1959 an informal conference was held for discussion of chronic lung diseases as possible antecedents to carcinoma of the lung. The conference brought together both experimental scientists and clinicians for consideration of the problem from these opposing directions of approach.

The stimulus of the session seems likely to bear fruit in the form of new research plans for attack on several phases of this problem.

Besides members of the Scientific Advisory Board, the following were present:

DR. CYRUS C. ERICKSON, Professor of Pathology, University of Tennessee, Memphis, Tennessee
DR. WALTER FINKE, Department of Medicine, The Genesee Hospital, Rochester, New York
DR. BURGESS L. GORDON, Director of Education, The Lovelace Foundation, Albuquerque, New Mexico
DR. HARRY S. N. GREENE, Professor of Pathology, Yale University School of Medicine, New Haven, Connecticut
DR. CECILIE LEUCHTENBERGER, Senior Biologist and Cytochemist, Children's Cancer Research Foundation, Boston, Massachusetts
DR. AVERILL A. LIEBOW, Professor of Pathology, Yale University School of Medicine, New Haven, Connecticut
DR. BJARNE PEARSON, Professor of Pathology, Wayne State University College of Medicine, Detroit, Michigan
DR. SHELDON C. SOMMERS, Pathologist, Massachusetts Memorial Hospitals, Boston, Massachusetts
DR. DOUGLAS H. SPRUNT, Professor of Pathology, University of Tennessee, Memphis, Tennessee
DR. ARTHUR J. VORWALD, Professor and Chairman, Department of Industrial Medicine and Hygiene, Wayne State University College of Medicine, Detroit, Michigan
DR. RUSSELL W. WELLER, Director of Laboratories, Ephrata Community Hospital, Ephrata, Pennsylvania

III. Carcinogenicity and Bioassay

1. ***Skin painting.***

Many efforts have been made by various investigators to determine,

through animal experiment, whether cigarette smoke has any direct cancer-producing activity. The most common type of test has consisted of painting smoke condensates on the skins of animals, especially mice.

Widely differing results have been obtained by different researchers. A few have reported cancers resulted on skins of some of their test mice after very long painting with high concentrations of smoke condensates. Others have reported that no skin reactions occurred.

One investigator has painted Swiss mice with cigarette smoke condensate, with and without mechanical irritation and with and without irradiation with X-rays. Some benign and malignant skin tumors were produced in all groups so painted regardless of coincident irritation. In Swiss female and C_3H male mice, intense irradiation of a small, distant skin area increased the sensitivity to smoke condensates.

The interpretation of these experiments in terms of their meaning for humans is very difficult for several reasons: (a) the methods of smoke production have often been such as to raise doubts whether the chemical composition is comparable to that of smoke produced in normal human smoking; (b) the differences in susceptibility of mice and people are so great that no quantitative conclusions can be drawn from these mouse experiments concerning hazards for man from smoke inhalation; (c) the dosages used in treating mouse skins are so great that they bear no realistic relation to the kinds of smoke dosages actually encountered by people in ordinary smoking, and (d) the lung has so much more efficient cleaning mechanisms than the skin that direct contact of smoke constituents with the lung is relatively brief.

2. *Other Methods of Bioassay.*

Because of the uncertainty of mouse skin painting as a test for the cancer activity of a substance, numerous other methods have been explored for a quicker, more reliable, and more easily interpreted test. A test that would be meaningful in terms of human experience would be a valuable contribution not only in the continuing tobacco research, but for all the other substances to which people are exposed in their day-to-day living. If, as has been suggested, we live in a "sea of carcinogens," it is extremely important to find a way to determine the identities and quantities of those substances that may have an effect on people.

Two scientists have sewn pellets of tobacco into the cheek pouches of hamsters for long periods of time but no cancers have been produced.

The same investigators have studied the suppression of the sebaceous glands of mice by carcinogenic hydrocarbons. It has been claimed by others that a parallel exists between the carcinogenic potency of such compounds

and their ability to suppress these glands in the skins of mice. An extensive study, however, showed the test to be low in power of discrimination. Moreover, the test was unreliable because some substances known to be carcinogenic gave negative results and vice versa. An effort to rate tobacco smoke condensate by this test gave an index number of 20 for the smoke from a whole carton of cigarettes as compared to a rating of 1000 for the weakest known carcinogenic hydrocarbon tested. On this basis, cigarette smoke would be considered virtually negligible in activity in terms of this test.

In another study, Sprague-Dawley male rats were fed diets containing 2-acetylaminofluorene to induce precancerous changes in the liver. One group was exposed to inhalation of cigarette smoke and another was injected with a laboratory preparation of smoke condensate (probably abnormal in composition) prepared by burning cigarette tobacco in a glass funnel. Both tobacco smoke exposures seemed to delay somewhat the appearance of precancerous liver changes.

Similar experiments with a smoke preparation from a carefully regulated machine that closely duplicates human smoking were made by injecting the preparation into, or painting it on, male albino rats on diets containing either 2-acetylaminofluorene or 3'-methyl-4-dimethylaminobenzene. This condensate had no effect on the rate or incidence of liver tumors in rats fed either substance.

Differences in Smoke Condensates

These findings emphasize the differences in various smoke condensates and illustrate the necessity of using smoke produced under normal conditions.

Other investigators have studied the possible co-carcinogenic effects of nitrogenous compounds from tobacco on (1) the incidence of spontaneous pulmonary adenomas in Strain A mice, (2) on urethane-induced pulmonary adenomas in Strain A mice, (3) on X-ray-induced lymphomas in C57 black mice, and (4) on ultraviolet light-induced skin tumors in Swiss mice. No such effects were found.

They have found that injection of finely divided carbon particles into the bloodstream will greatly increase the number of lung tumors that normally occur in Strain A mice. A neutral fraction of tobacco smoke adsorbed on such carbon particles before injection slightly *decreased* the number of tumors produced by the carbon.

It was also found that a niacin-deficient diet in Strain A mice will greatly increase the incidence of spontaneous lung adenomas, whereas a supplement of niacin seems to be protective. Feeding 3-pyridyl methyl ketone, an oxidation product of nicotine that conceivably could act as a niacin antimetabolite, appeared to alleviate the niacin deficiency and de-

crease the tumor incidence. The possible action of whole inhaled smoke as an anti-niacin agent is now under study.

Another scientist has explored several methods that might develop into useful bioassay techniques for measuring carcinogenicity. Using a subcutaneous airpouch for tumor transplantation, he has measured the effects of known carcinogenic agents in enhancing or inhibiting growth. Tobacco smoke condensates so far employed have shown no action. Some complications have developed in the application of the method and it has not yet evolved into a wholly satisfactory test procedure.

The uterine cervix of mice has been used as a test tissue for determining the potency of agents suspected of being carcinogenic. This tissue resembles lung tissue more closely than does the skin. Cigarette smoke condensates were applied to the cervix and upper vaginal area of more than 200 DBA1 mice over a period of many months. Controls received solvent alone, methylcholanthrene in the same solvent, or swabbing with a dry cotton tipped applicator. These mice proved to be too sensitive to produce a valid test since the solvent alone or the dry applicator resulted in more tumors than did the application of the smoke condensates.

In view of these results, it has been necessary of course to abandon this method as a valid test. While others have considered significant the induction of tumors at this site with smoke condensates, the higher incidence of tumor induction with a plain applicator in this work raises serious doubt as to the validity of any such conclusions.

Carcinogenesis in Lymph-less Animals

Extensive studies have been made of carcinogenesis in ducks and chickens by methylcholanthrene applied to various tissues. The responses of animals equipped with feathers instead of fur and lacking lymph glands were considered worth studying for contrast with the more familiar responses of mammalian species. Many interesting observations on the induction of tumors and on their behavior have been recorded. Most relevant perhaps is the contrast in action of methylcholanthrene introduced into the trachea in peanut oil solution and that of tobacco smoke condensates introduced in the same vehicle. The methylcholanthrene quickly produces a very intense inflammation while the cigarette smoke is practically without effect.

Another investigator is studying still another phenomenon that appears promising as a basis for a test method. He has observed that certain tumors which cannot ordinarily be transplanted to a strain of mice in which they are alien, will "take" and grow if the animals are first painted (or fed) with carcinogenic hydrocarbons for a period of time prior to the

transplant. The time during which the transplant will grow is related to the dosage of hydrocarbon given. With sufficient dosage, even normal skin from the alien strain can be transplanted successfully for periods related to the dosage. This method appears to be well suited to quantitation and its evaluation by comparison of a large collection of polynuclear hydrocarbons is under way.

One scientist has undertaken to guide further study of a carcinogenicity assay method based on the response of a protozoan *(Paramecium cordatum)* to ultraviolet light following exposure in the dark to a polynuclear hydrocarbon. The response is reputed to be related to carcinogenic activity of the hydrocarbon. Comparison of a large number of such hydrocarbons is to be made in parallel with tests by the method described in the preceding paragraph.

Bioassay Conference

A conference was held September 10, 1959 for discussion of carcinogenesis and bioassay methods. Critical analysis was made of hitherto prevalent methods and of recent studies suggesting new approaches to the problem. The discussion resulted in the planning of several new coordinated studies to compare the results of different assay techniques.

Besides members of the Board, the following were present:

DR. R. S. BECKER, Associate Professor of Chemistry, University of Houston, Houston, Texas
DR. R. K. BOUTWELL, Associate Professor of Oncology, University of Wisconsin Medical School, Madison, Wisconsin
DR. GIUSEPPE DELLA PORTA, Division of Oncology, The Chicago Medical School, Chicago, Illinois
DR. LOUIS F. FIESER, Professor of Chemistry, Harvard University, Cambridge, Massachusetts
DR. FREDDY HOMBURGER, President, Bio-Research Institute, Inc., Cambridge, Massachusetts
DR. A. W. HORTON, Associate Professor of Industrial Health, University of Cincinnati College of Medicine, Cincinnati, Ohio
DR. MAURICE LANDY, Head, Polysaccharide Section, Laboratory of Chemical Pharmacology, National Cancer Institute, Bethesda, Maryland
DR. MELVIN S. NEWMAN, Professor of Chemistry, Ohio State University, Columbus, Ohio
DR. WILLIAM E. POEL, Research Associate in Charge, Laboratory for Experimental Carcinogenesis, University of Pittsburgh Graduate School of Public Health, Pittsburgh, Pennsylvania
DR. BENJAMIN A. RUBIN, Assistant Professor of Epidemiology, Baylor University College of Medicine, Houston, Texas
DR. MURRAY J. SHEAR, Chief, Laboratory of Chemical Pharmacology, National Cancer Institute, Bethesda, Maryland
MISS JOAN SMITH, (Representing Dr. T. M. Sonneborn, Department of Zoology, Indiana University, Bloomington, Indiana)

DR. PAUL E. STEINER, Pathologist, The Institute for Cancer Research, Philadelphia, Pennsylvania
DR. C. S. STEPHANO, Director of Research, Stephano Brothers, Inc., Philadelphia, Pennsylvania
DR. JOHN C. SZERB, Associate Professor of Pharmacology, Dalhousie University, Halifax, Nova Scotia

IV. Tissue Culture

In the hope that the observation of human lung tissue grown *in vitro* and study of its responses to carcinogenic and other agents might provide new understanding of the nature of carcinogenesis, the culture of such tissue has been undertaken.

Grants have been made to four laboratories for (1) training personnel in this kind of work, (2) attempting to establish strains of various human tissues, especially lung tissues, (3) observing the nature of changes from normal to malignant cells, and (4) observing and describing the responses of such tissue to tobacco smoke ingredients and other agents.

V. Cardiovascular Research

The Scientific Advisory Board has continued its active interest in the field of cardiovascular research with special reference to the effect of nicotine and smoking on the circulation. Research grants have been made by the T.I.R.C. to support work in 16 centers concerned with cardiovascular physiology and its disorders.

The 1958 Report of the Scientific Director contained descriptions of the scope and, when available, the results of many projects supported by the T.I.R.C. Since then, a number of publications have appeared in the scientific literature and are abstracted in this Report. Some deal with basic problems in cardiovascular physiology while others are concerned with changes induced by nicotine and the smoking of cigarettes.

In view of the speculation about heavy smoking and deaths from coronary disease, a brief review of evidence, relating to the effect of nicotine and smoking on the coronary circulation and based on the results reported by T.I.R.C. grantees, might be of special interest.

Differences In Response

Since smoking or the injection of nicotine commonly results in a reduction in the superficial circulation, as indicated by plethysmographic measurements of the hand or by a fall in skin temperature, many have considered nicotine to be a general vasoconstrictor agent. This overlooks the fact that there are great differences in the response of different vascular beds to many chemical agents. It has long been known that there is a recip-

rocal action between the vasomotor tone of the deep and superficial vessels. That is, when there is constriction in one area, as during anesthesia or asphyxia, there is dilatation in the other. This represents an aspect of homeostatic regulation through which the total peripheral resistance and the capacity of the vascular system remain constant. Thus the cardiac output and blood pressure may be little affected in the face of relatively large changes in peripheral resistance or those originating in either the deep or superficial vasculature.

There is general agreement that, in the isolated heart, nicotine added to the perfusing medium results in dilatation of the coronary arteries. However, it was not justified to assume from this observation that such is the case in the intact animal, since nicotine affects other structures that may secondarily influence the coronary blood flow. Thus the effects of epinephrine on the circulation resemble in many respects those of nicotine, and since nicotine stimulates the adrenal medulla, it is probable that the epinephrine released contributes to the changes observed.

There is a fairly extensive literature on the effect of smoking on the coronary circulation. Much of the work on which this is based is not properly controlled and need not be described here. However, recent studies in both man and other mammals leave no doubt that nicotine and cigarette smoke result in an increase in coronary blood flow. The significance of this needs further consideration since there is also an increase in heart rate and in the force of contraction due, at least in part, to a direct action of nicotine on intracardiac structures.

In view of the fact that nicotine increases cardiac work, the question arises as to whether, under these circumstances, the circulation keeps pace with the metabolic requirements of the heart. In dogs, intravenous infusions of nicotine and direct injection into the coronary arteries resulted in a definite increase in coronary flow. In these experiments there was a corresponding increase in cardiac work, but because in some instances there were changes in the A-V/oxygen difference and inversion of the T-wave, there may have been some interference with the utilization of oxygen — a point deserving of further study.

Complex Action of Nicotine

The question of practical concern is the effect of smoking on coronary flow in man. The actions of nicotine are complex and include stimulation or depression of various parts of the nervous system and peripheral structures such as the muscles and glands. Hence, it is important to determine what happens to the coronary circulation under normal conditions of smoking. Experiments have been carried out utilizing the technique of

inserting a catheter directly into the coronary sinus. The data obtained showed that smoking results in a significant increase in the coronary blood flow and a significant decline in the coronary vascular resistance. There was a fall in myocardial oxygen extraction, but since this was proportional to the rise in coronary blood flow, it was concluded that the oxygen consumption was not significantly changed.

The experiments just mentioned are in accord with the observation that electrocardiographic or ballistocardiographic changes are not seen in healthy individuals as a result of smoking. However, when the coronary arteries are diseased, smoking commonly produces changes in the electrocardiogram characteristic of anoxia, and this has been employed as a test of coronary function. There is also the fact that in an occasional patient with angina pectoris an attack of pain may be precipitated by smoking or other activities. These observations suggest that the effects of smoking on the heart may not always be the same in health and disease.

This is an important problem for further investigation. Since some risk may be involved in cardiac catheterization in the presence of coronary disease, other techniques for the measurement of blood flow are under study. In the meanwhile a start has been made on laboratory studies in which the effects of nicotine have been tested in rabbits made atherosclerotic by high cholesterol diets. It has been reported that in the normal heart, perfusion with nicotine resulted in a brief decrease in coronary flow that gave way to a more prolonged increase in the rate of flow. The latter phase, representing a dilatation of the coronary arteries, was absent in the atherosclerotic heart.

In another study of rabbits maintained on a high cholesterol diet, the oral administation of nicotine did not affect the serum cholesterol and phospholipid values or the degree of aortic atherosclerosis, but did result in an increased mortality and increased incidence of pathological changes in the heart and peripheral vasculature. Similar experiments in chickens, but with larger doses of nicotine, produced no significant cardiovascular pathologic changes over those resulting from the high cholesterol diet alone. The results of further studies along these lines are awaited with special interest.

Cardiovascular Conference

Grantees and other scientists doing research in the cardiovascular field met for a general discussion June 12, 1959. In addition to members of the Scientific Advisory Board, the following were present:

DR. ERNST O. ATTINGER, Boston City Hospital, Boston, Massachusetts

DR. KURT DE CRINIS, Goldwater Memorial Hospital, Welfare Island, New York, N.Y.
DR. FRANK W. DAVIS, JR., Johns Hopkins University School of Medicine, Baltimore, Maryland. (Member, Committee on Smoking in Relation to Cardiovascular Disease, American Heart Association)
DR. THOMAS R. DAWBER, National Heart Institute, Bethesda, Maryland. (Member, Committee on Smoking in Relation to Cardiovascular Disease, American Heart Association)
DR. JACK FREUND, Medical College of Virginia, Richmond, Virginia
DR. JAMES GILLETTE, Laboratory of Chemical Pharmacology, National Heart Institute, Bethesda, Maryland
DR. HARVEY B. HAAG, Medicall College of Virginia, Richmond, Virginia
DR. PAUL S. LARSON, Medical College of Virginia, Richmond, Virginia
DR. ANTONIO DE LEON, Philadelphia General Hospital, Philadelphia, Pennsylvania
DR. KELLY T. McKEE, Medical College of South Carolina, Charleston, South Carolina
DR. HERBERT McKENNIS, Medical College of Virginia, Richmond, Virginia
DR. EDWARD W. PELIKAN, Boston University School of Medicine, Boston, Massachusetts
DR. WALTER REDISCH, New York University—Bellevue Medical Center, New York, N.Y.
DR. SEYMOUR H. RINZLER, Cornell University Medical College, New York, N.Y.
DR. JERRY E. SCHMITTHENNER, Lankenau Medical Building, Philadelphia, Pennsylvania
DR. CAROLINE BEDELL THOMAS, Johns Hopkins University School of Medicine, Baltimore, Maryland
DR. JANET TRAVELL, Cornell University Medical College, New York, N.Y.
DR. DUANE G. WENZEL, University of Kansas, Lawrence, Kansas
DR. J. EDWIN WOOD, Medical College of Georgia, Augusta, Georgia

VI. Psycho-physiological Studies

During the course of the past year several new studies were inaugurated in the fields of psychology or of psycho-physiology.

One undertaking is to study smoking practices of young college students in relation to family history, type of secondary schooling, academic interests, academic achievement, and social relationships.

Another group is exploring the behavioral effects of smoking under stress conditions to determine whether such effects are comparable to those of tranquilizing drugs.

Body morphology in relation to tobacco use is now the subject of a special investigation.

A pilot study of smoking habits among older persons is being made through the Age Center of New England.

VII. Miscellaneous

Several studies bearing on the cancer problem are summarized here.

One scientist has been investigating the storage of various trace metals in human lungs and livers in search of possible clues to the prevalence of particular diseases in certain localities.

Further investigation has been made of how carcinogenic azo dyes are incorporated into certain protein constituents of the liver.

A scientist has made a basic study of the mechanism of hyperplasia and resistance. He has shown how irritation will first produce protein depletion of a tissue as reflected in circulation of cellular proteins as globulins in the serum, then increased resistance to further damage. As resistance increases, the initial hyperplastic response falls off sharply and the resistant skin loses its hyperplasia. Skin responds to tobacco smoke much as it does to other agents.

A basic study was made of the pathology of human oral tissues, especially as related to aging.

Old chest X-ray plates of persons who were eventually diagnosed as having lung cancer were studied. The main purpose was to improve the interpretation of the earliest changes suggestive of lung cancer as revealed by radiology. As a by-product of this study, an analysis was made of the occupations of the persons whose histories were collected. This showed that persons whose occupations subjected them to inhalation of dusts and fumes were more likely to be heavy smokers than persons not so exposed.

Analytical studies were made to compare the traces of polynuclear hydrocarbons in mainstream and sidestream smoke produced under various conditions. The effects of smoke on the ciliary activity and mucous flow in isolated bronchi of several animal species also were observed.

Fellowship Program

The medical student fellowship program, originated in 1955 upon recommendation of the Scientific Advisory Board and renewed each year since, continued to show encouraging results in 1959.

The program is designed to stimulate interest in basic research among medical school students who, with their advisors, decide on the subjects for study.

Recipients of the fellowships are selected by the deans of the medical schools for work in the summer or other off-term time in the laboratories of experienced scientists. Students who have participated in the program have written papers on their research, and some of these have been published or submitted for publication in journals.

Abstracts of Reports

Each recipient of a Tobacco Industry Research Committee grant is responsible for the initial presentation or publication of the results of his research in scientific meetings or in appropriate scientific journals.

Following are abstracts, approved by the authors, of research reports with acknowledgement of support from the T.I.R.C. that have appeared in scientific journals since the last Report and through July 1959. Abstracts of earlier papers by grantees which appeared in previous reports of the Scientific Director are listed at the end of this section. The name of the scientist to whom the T.I.R.C. grant was made is given in parenthesis where required.

These abstracts have been grouped under five headings: I. Cancer Research; II. Human Lung Studies; III. Heart and Circulation; IV. Tobacco Chemistry; and V. Other Studies.

I. *Cancer Research*

"TISSUE SPECIFICITY OF SERUM COMPONENTS." By S. D. YEH, M.D., W. F. SEIP, B.S., C. BURCH, A.B., and F. W. BARNES JR., M.D., with the aid of R. RUTHERFORD, M.D., Departments of Medicine and Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Md. *A. M. A. Archives of Internal Medicine,* Volume 103, pages 933-948, June 1959. (T.I.R.C. grantee: Barnes)

Purpose of study: Serum proteins incorporate amino acids very rapidly, and it seems justifiable to conclude that this process takes place within cells. Under a hypothesis derived from a theory of cell response to damage evolved in this laboratory, the serum globulins have a cellular origin from a large variety or perhaps all of the tissues of the body; they escape and circulate in the body fluids, and then reenter their respective cells of origin to resume the role in protein metabolism in which they were involved before leaving the cell. Experiments were devised to test the theory that the components of normal human serum possess tissue specificity.

Procedures: Tissues from human organs (kidney, muscle, skin, thyroid) were obtained freshly in the operating room when possible; and human heart, brain, liver, spleen, lymph node, aorta and connective tissue were obtained post mortem. For injection into rabbits, the material was used first as a suspension in saline and later in the form of a centrifuged supernatant of the suspension. Immunization of the rabbits proceeded until an adequate level of antibodies was shown. Cross reactivity was removed for certain ranges of dilution of antigen and antiserum. The precipitin test was used to estimate the possible limits of unknown serum or of antigen dilution and also to approximate the order of magnitude of the antigen-antibody precipitate. Appropriate control tests also were conducted.

Findings: The results show that the antisera prepared from another species, with relative specificity for components of a single human organ, will fractionate parts of the serum proteins of individual persons by production

of precipitins. The results are reproducible; also each person at various times has a different pattern of precipitin fractions, measured by limit of serum dilution. These facts indicate that serum proteins are certainly not homogeneous but can be divided into many independent fractions by anti-tissue sera, and help to confirm the concept developed from work on tissue damage that proteins derived from many tissues circulate as serum proteins. The results also provide further evidence that tissue specificity does exist in the mammal.

Other Grantors: American Cancer Society (National and Maryland Division), American Heart Association, National Institutes of Health, Damon Runyon Memorial Fund, Milton M. Frank Foundation, and the Noxzema Corporation.

"TUMOR-HOST RELATIONS DURING DEVELOPMENT AND AFTER REGRESSION OF THE TUMOR." By LEOPOLD R. CERECEDO, EDWARD BRESNICK and EDWARD T. SCHUBERT, Department of Biochemistry, Fordham University, New York City. (Dr. Bresnick is now at the Wellcome Research Laboratories, Tuckahoe, N. Y.). *Archives of Biochemistry and Biophysics*, Volume 83, pages 44-53, July 1959. (T.I.R.C. grantee: Cerecedo)

Purpose of study: Previous studies in this laboratory have shown that significant changes occur in the weight and also in the nucleic acid concentrations of specific tissues during the growth of tumors in the rat, and that the presence of subcutaneous tumors causes increases in the deoxyribonucleic acid (DNA) concentration of the liver and lung, whereas concentration of ribonucleic acid (RNA) may or may not change, depending on the type of tumor. In view of the nature and distribution of potassium and its importance in cellular metabolism, its levels were found to be related to other phenomena characteristic of neoplastic growth. It was decided to examine these factors in connection with the growth and regression of various neoplasia in rats and mice.

Procedures: The Murphy-Sturm lymphosarcoma was transplanted in the pectoral region of male Holtzman rats by means of a trocar and the Jensen sarcoma in Blue Spruce Farms rats. Suspensions of C1498 myeloid leukemia cells were inoculated in the right pectoral region of male C57B1/B10.D2 mice. Livers, lungs, kidneys and spleens were extirpated after the animals were sacrificed, and specimens of bone marrow and blood also were taken. Chemical and physiological changes during development and after regression of the tumors were studied.

Findings: Growth of the lymphosarcoma in the rat was accompanied by an increase in DNA content of liver, lung and spleen and a decrease of DNA in bone marrow; of the tissues mentioned, spleen alone showed a change (increase) in concentration of RNA; the potassium concentration of liver also rose. Concentration of DNA in the lung rose during the development of the Jensen sarcoma, while RNA remained unchanged; hemoglobin, specific gravity, and total solids of the blood were depressed markedly, and attained minimum levels when tumor size reached 20% of the body weight. The leukemia had no effect on the DNA of liver or kidney in the mouse, but induced a rise in lung DNA and a decrease in DNA of the spleen. No changes in RNA appeared in any of these tissues.

When the three afore-mentioned tumors regressed, each of the changes described was reversed and normal patterns were re-established.

Other grantor: National Cancer Institute.

"ACTION OF CIGARETTE TAR AND SMOKE ON CHEMICALLY INDUCED CARCINOGENESIS." By HOWARD E. HOFFMAN and A. CLARK GRIFFIN, Department of Chemistry, Stanford University, California. Present addresses: Stine Laboratory, E. I. du Pont de Nemours & Co., Newark, Delaware, and Department of Biochemistry, University of Texas M. D. Anderson Hospital and Tumor Institute. *Texas Reports on Biology and Medicine,* Vol. 16, pages 333-345, Fall 1958. (T.I.R.C. grantee: Griffin)

Purpose of study: As certain weak carcinogens or non-carcinogens have acted both as synergistic and as antagonistic agents in animal experiments, it was considered desirable to determine whether cigarette smoke or condensate would enhance or inhibit the course of a well-established carcinogenic sequence, namely that resulting from feeding to rats a diet containing liver carcinogens.

Procedure: In the first experiments two groups of Sprague-Dawley male rats were fed diets containing DAF (2-acetylaminofluorene) for periods of two and three months. One group was exposed to the smoke of standard filterless cigarettes smoked in a machine at the rate of 20 cigarettes an hour, 8 hours daily, for 12 to 14 weeks. In subsequent tests the rats received smoke at the rate of 5 cigarettes an hour, 8 hours daily. The other group of rats was injected on alternate days with 0.1 ml. of a condensate prepared by burning cigarette tobacco in a glass funnel; the condensate was suspended in peanut oil at a concentration of 50 mg./ml. Controls received peanut oil only.

In the second experiments cigarette smoke condensate, commercially prepared in a smoking machine closely duplicating human smoking, was injected in male albino rats on diets containing either DAF or 3'-MeDAB (3'-methyl-4-dimethylaminobenzene). Other rats on the same diets were painted twice weekly with the same condensate on shaved areas on the back of the neck.

Findings: Inhalation of cigarette smoke and injection of the laboratory cigarette condensate delay the appearance of the precancerous state in rats fed DAF, as seen in gross damage to the liver and in delay of riboflavin lowering of precancerous liver. No protective action of laboratory condensate was observed in rats fed 3'-MeDAB, however. Administration of the commercially prepared cigarette smoke condensate to rats fed either carcinogen did not affect the rate of cancer induction or the final tumor incidence. It must be recognized that different smoke tar fractions vary considerably in composition depending upon the conditions of combustion and other factors.

"HYALURONATE-INACTIVE SPREADING FACTOR OF MURINE ENDOMETRIAL SECRETIONS (UTERONE)." By F. HOMBURGER, M. S. GROSSMAN, and P. C. HARPEL, Bio-Research Institute, Inc., Cambridge, Mass., and Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me. *Proceedings of the Society for Experimental Biology and Medicine,* Vol. 99, pages 665-668, December 1958. (T.I.R.C. grantee: Homburger)

Purpose of study: The experiments were designed to measure the effect of uterone in spreading India ink in the dermis of test animals and to elucidate its mechanism. This research is intended to clarify observations made during studies of biologic activities of endometrial secretions (uterone) obtained by cervical ligation in mice, which revealed that crude uterone enhances intradermal spread of India ink.

Procedure: India ink was injected intradermally in groups of mature male and female RFM and BALB/c mice. Six groups comprising 27 RFM males received India ink marker containing 6U, 12U, 50U and 100U of hyaluronidase, (Wydase), and the spread was compared with that resulting from serum or Ringer's solution. Five RFM males received India ink and fresh uterone from mice of the same species, and the spread was compared with that in mice receiving India ink and serum. Effects of uterone from BALB/c mice were measured in 19 male and 15 female mice of that species given India ink marker and uterone in five different concentrations, and the spread was compared with India ink and serum injections. Eight BALB/c males were given India ink and hyaluronidase dissolved in uterone from mice of the same species. Further tests were made in dead BALB/c mice skin. Control experiments were conducted in rabbits, the standard assay animal for the spreading factor.

Findings: The results indicate that commercial Wydase enhances the spreading of India ink in the dermis of mice much as it does in the rabbit. Intradermal injection of murine uterone from BALB/c and RFM mice enhances spreading of India ink in live mice of these species. BALB/c uterone potentiates the spreading effect of Wydase in live BALB/c mice, but is without effect upon injection into dead BALB/c skin. BALB/c uterone does not depolymerize hyaluronic acid *in vitro*. The potentiation of Wydase by uterone could be explained by increase of intra-dermal pressure caused by inflammation and ensuing wider diffusion of the enzyme, hence a greater spread. The nature of the component of uterone responsible for provoking inflammation remains to be determined and may well be a mucoprotein.

"KERATOACANTHOMA EXPERIMENTALLY INDUCED WITH METHYLCHOLANTHRENE IN THE CHICKEN." By R. H. RIGDON, M.D., Professor of Pathology, University of Texas Medical Branch, Galveston. *A.M.A. Archives of Dermatology,* Vol. 79, pages 139-147, February 1959.

This paper discusses the similarity of a squamous cell tumor produced in the skin of chickens after local application of methylcholanthrene to the keratoacanthoma in man.

"THE RESPIRATORY SYSTEM IN THE NORMAL WHITE PEKIN DUCK." By R. H. RIGDON, Department of Pathology, University of Texas Medical Branch, Galveston. *Poultry Science,* Vol. 38, pages 196-210, January 1959.

"MECHANISM OF REMOVAL OF FLUID AND PARTICULATE MATERIAL FROM THE RESPIRATORY TRACT OF THE DUCK." By R. H. RIGDON, *A.M.A. Archives of Pathology,* Vol. 67, pages 215-227, February 1959.

Purpose of studies: Anatomical findings noted in the study of induced

carcinogenesis in the respiratory tract of the white Pekin duck made it necessary to establish the "normal" for this bird. While the anatomic structure of the respiratory system is similar in many ways to that of mammals, there are certain fundamental differences that suggest a possible variation in function. Little attention has been given to the mechanism of the removal of fluid and particulate matter from the respiratory tract of birds.

Procedures: In the first study latex was injected to produce moldings of the respiratory tracts of ducks. To study blood capillaries in the lung, an adult bird was sacrificed and saline injected immediately into the pulmonary artery; when the saline returned from the pulmonary veins free of red cells, India ink was injected into the pulmonary artery; after the ink had completed a transit, sections of lung were prepared for histologic study.

In the second study isotonic saline, fluorescein sodium, saccharated iron oxide, India ink, liquid petrolatum, and Lipodium spores were given intratracheally to separate groups of ducks, which were sacrificed after varying intervals of exposures. The thoracic and abdominal viscera and the air sacs were observed under ultraviolet light for fluorescence in the lungs, air sacs, liver, gallbladder and intestines. The trachea and lungs subsequently were removed intact, and sections were taken for histologic study.

Findings: There are no lymph nodes in the duck, unlike mammals. There are lymph channels in the lung which drain into the larger lymphatics, which in turn communicate with the thoracic duct and then empty into the vena cava. When particulate matter is put into the trachea, it usually can be demonstrated in the dilated parabronchi at the periphery of the lung and in the larger air sacs. The cilia on the epithelial cells in the respiratory tract degenerate rapidly following death, so care must be exercised in expressing an opinion on the significance of the absence of cilia from the wall of the trachea.

In the second study, saccharated iron oxide and fluorescein sodium passed directly through the epithelial layer of the respiratory tract into the stroma and through the wall of the blood vessels and/or lymphatics. Particles of India ink, globules of petrolatum, and Lipodium spores migrated through the layer of epithelium and either entered the blood vessels and/or lymphatics directly or were phagocytized within the stroma by fibroblasts and histocytes. The transfer of fluids and particulate matter from the respiratory tract to the vascular system occurred primarily in the air sacs.

"CANCER OF THE LUNG — THE SEX RATIO. A REVIEW OF THE PROBLEM." By HELEN KIRCHOFF and R. H. RIGDON, Department of Pathology, University of Texas Medical Branch, Galveston. *Texas Reports on Biology and Medicine,* Vol. 17, pages 29-48, Spring 1959. (T.I.R.C. grantee: Rigdon)

The available literature on the sex ratio of cancer of the lung between 1871 and 1957 has been reviewed. The male to female ratio in 108 studies (17,609 cases of lung cancer) varied from 0.7:1 to 20.9:1, with the average ratio five male cases to one female case.

From the available data it appears that the increase in lung cancer has been greater in the male than in the female. However, the question may be asked — how much of this difference is real and how much is only apparent? This review shows the state of confusion referable to the incidence

of lung cancer in the male and female.

Data are available to show that the male-female "mortality rates" for lung cancer were at about the same level in 1914; by 1930 they were 1.7:1 and in 1950 had increased to 4.6:1 (Cutler, J. Am. Stat. Assn. 50:267-282, 1955). In reviewing all the data, it does not appear correct to accept this ratio of 1.7:1 as a starting point. It would appear from this review that the male-female ratio of lung cancer was approximately 3 or 4 to one in 1900 and 5 or 6 to one in 1955.

"TYPES OF KERATINIZATION IN THE HUMAN GINGIVA." By J. P. WEINMANN, M.D., and J. MEYER, Ph.D., Division of Oral Pathology, University of Illinois, College of Dentistry, Chicago. *Journal of Investigative Dermatology*, Vol. 32, pages 87-94, February 1959. (T.I.R.C. grantee: Dr. Isaac Schour)

Purpose of study: In studying the epithelial surface in the human gingiva, four types of staining reaction were found. Three correspond to the previously recognized distinctions of full keratinization, parakeratosis, and non-keratinization, which are considered as normal variants of the gingiva. The fourth has not been described hitherto, and the term "incomplete parakeratosis" is suggested for it. In order to clarify the conditions in which these types of keratinization occur, it is necessary to subdivide the gingiva into anatomically distinct regions.

Procedure: Biopsy specimens from the cuspid region of gingiva of 52 men 27-70 years of age were studied microscopically. Glycogen concentration, estimated as the difference in PAS positive material without and with diastase treatment, and the degree of inflammation in the underlying connective tissue, were classified as O, slight, and concentrated or severe. The analysis included crest and oral surface of the free gingiva and the attached gingiva — the portion in part attached to the tooth and in part to the buccal or lingual surfaces of the alveolar bone.

Findings: The type of keratinization was ascertained in a total of 234 regions of the gingiva. The relations between degree of keratinization and the presence of glycogen and inflammation differ quantitatively, but not qualitatively, in the crest, oral, and attached areas. The distinction of "incomplete parakeratosis" — made possible by very careful staining techniques — permitted separate study of about a third of the parakeratotic regions. Parakeratosis still remained the single most frequent type of keratinization, occurring in a little over half of the sample.

Other grantor: National Institutes of Health

II. *Human Lung Studies*

"LUNG VOLUME IN SMOKERS AND NON-SMOKERS." By HENRY BLACKBURN, M.D., JOSEF BROZEK, Ph.D., and HENRY L. TAYLOR, Ph.D., Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis. *Annals of Internal Medicine*, Volume 51, pages 68-77, July 1959. (T.I.R.C. grantee: Brozek)

Purpose of study: Tobacco smoking has long been suspected as an etiologic factor in respiratory symptoms and chronic bronchopulmonary disease, largely on the basis of clinical impression. It was considered that measurement of anatomic lung compartments in older individuals might be

expected to provide some information concerning ultimate lifetime effects of smoking on bronchopulmonary function.

Procedure: Data were collected for 221 business and professional men of the Twin Cities aged 47 to 57 who participated in a 10-year longitudinal study of cardiovascular aging. All were actively employed and were free of manifest heart disease, hypertension, and gross parenchymal lung involvement. All filled out a questionnaire on smoking habits comparable to that of Hammond—Horn of the American Cancer Society. Vital capacity of each subject was determined at rest, and residual air volume was determined after forced expiration with the subject submerged in water in a sling seat; two or more preliminary tests were made to "train" the subject so that minimal values of residual lung volume might be obtained. After the final immersion, the subject was raised and breathed O_2 for seven minutes. Residual volume and nitrogen excretion were then measured. Age and body size were eliminated as variables in the lung volume comparisons.

Findings: Vital capacity was smaller in each category of smokers. The difference from non-smokers reached a statistically significant level for all smoking categories combined. Residual lung volume was larger in all categories of smokers, but not consistently at statistically significant levels. Total lung capacity was not significantly different between groups. The ratio of residual volume to total lung capacity was significantly greater in smokers.

In a group which had successfully stopped all smoking, the lung compartment values were similar to those of a group who had never smoked, and the ratio of residual volume to total lung capacity was significantly smaller than in the current smokers. Stringent screening of the sample for abnormal respiratory manifestations, such as histories of clinical asthma, pulmonary tuberculosis, chronic productive cough, and chest deformity, excluded 33 subjects (22 smokers and 11 "Never" or "Stopped" smokers): this left a residual of 188 men in whom the same order of differences in lung volume prevailed between smokers and non-smokers as in the total sample.

The differences recorded were in the direction to be expected if smoking is a factor in producing a functional increase of airway resistance.

Other grantor: National Heart Institute

"THE EFFECTS OF SMOKING ON THE RESPIRATORY SYSTEM IN NORMAL INDIVIDUALS." By KELLY T. MCKEE, Department of Medicine, Medical College of South Carolina, Charleston. *Southern Medical Journal,* Vol. 51, pages 1110-1115, September 1958.

Purpose of study: Because of the somewhat conflicting conclusions drawn from a number of studies measuring various aspects of pulmonary function, it seemed worthwhile to study a group of normal individuals, smokers and non-smokers, to determine whether any significant difference in easily measured ventilatory components of breathing could be observed in the two groups.

Procedure: Measurements of the vital capacity and maximum breathing capacity were carried out in 175 young men aged 20 to 41 (85% were 21-28), including 60 non-smokers; 71 of the smokers smoked between one and two packs of cigarettes daily and the remaining 44 one-half to

one pack daily. Duration of cigarette usage in the smoking group varied from one to 15 years. In 50 of the smokers the measurements were made before and immediately after smoking one cigarette, after a period of abstinence of four or more hours.

Findings: Slight but probably not significant decrease in total vital capacity in heavy smokers as compared to non-smokers was noted. A similar very slight decrease in maximal breathing capacity in heavy smokers as compared to non-smokers was noted. No trend toward a decrease in maximal breathing capacity with increasing years of cigarette usage was observed. It would appear, on the basis of these studies, that no evidence has been produced to show that ventilatory function is significantly impaired in young smokers as compared with non-smokers, nor is there any definite evidence that ventilatory function in these smokers differs appreciably after smoking a cigarette after several hours of abstinence from smoking.

"STUDIES ON THE NATURE OF HYPOXIA WITH AND WITHOUT CYANOSIS IN CHRONIC PULMONARY DISEASE." By HURLEY L. MOTLEY, M.D., Professor of Medicine and Director of the Cardiorespiratory Laboratory, University of Southern California School of Medicine, Los Angeles. *Geriatrics,* Vol. 13, pages 617-633, October 1958.

Purpose of study: This study was made to determine the nature of the arterial blood changes in patients, without emphysema, complaining of dyspnea and fatigue on exertion and without cyanosis. The arterial blood reflects the blood gas exchange in the lungs. Samples of blood from the femoral vein have been mistaken for arterial femoral blood, which may be dark in the presence of hypoxia.

Procedure: Fourteen men and six women aged 17 to 81 made up the study group; there were 14 cases of fibrosis of unknown etiology, two cases of sarcoidosis, three of asbestosis, and one of lupus erythematosus. In one fibrosis case there was associated agammaglobulin anemia and in another bronchiectasis. Pulmonary function measurements under varying test conditions consisted of lung volume, arterial blood, and pulmonary ventilation. The total lung capacity was decreased in all cases, and emphysema was not a factor.

Findings: These studies indicate that the alveolar-capillary membrane block is not a significant factor in the hypoxia of pulmonary fibrosis, emphysema, and in most of the related conditions, such as the collagen group of diseases. The true cause of this condition is primarily one of obstruction in which the air breathed does not get down to the alveolar level where the blood gas exchange occurs. This diagnosis has been missed in the past and incorrectly described because the graded levels of high oxygen breathing (32 to 40% oxygen) were not used, especially with exercise. Breathing 100% oxygen at rest constitutes an inadequate test by which to rule out shunting at the alveolar level.

Other grantor: National Institutes of Health

"THE NATURAL HISTORY OF CARCINOMA OF THE LUNG." By GEORGE L. EMERSON, M.D., MARION S. EMERSON, M.D., and CHARLES E. SHERWOOD, M.D., Departments of Medicine, Radiology and Surgery, The University of Rochester School of Medicine and Dentistry, Rochester, New

York. *Journal of Thoracic Surgery,* Vol. 37, pages 291-304, March 1959. (T.I.R.C. grantee: Sherwood)

Purpose of study: The principal objective has been to gain new information about early changes in the lungs preceding the diagnosis of carcinoma of the lung and, where possible, to observe the alterations in x-ray shadows, preceding the appearance of clinical symptoms, with the passage of time.

Procedure: Over 400 cases of pulmonary cancer seen in the University of Rochester Hospital were reviewed, covering 360 proved cases, the majority having been diagnosed within the past 15 years. Records were abstracted and information was solicited from surviving patients, families, physicians, hospitals, and other institutions where radiographs may have been taken. Any past radiographs available were gathered and reviewed in conjunction with later films made at the time of diagnosis of carcinoma. Certain ancillary statistical data were included for general interest, without further elaboration.

Findings: Radiographically, obstructive pneumonitis was present in 37% of the cases, parenchymal mass in 21%, hilar nodularity in 20%, hilar mass in 19%, atelectasis in 15%, mediastinal nodes or mass in 9%, pleural effusion in 8%, parenchymal fibrous infiltrate in 3%, and miscellaneous abnormalities in 9%. The average interval between first symptom and clinical diagnosis was seven months. The interval between the first radiographic evidence of cancer and its diagnosis was four months for all cases; this figure includes a large number where no x-rays were taken prior to the time of establishing the diagnosis. In the more significant group, where radiographs had been made six months or more prior to clinical diagnosis, this delay was 16 months.

The isolated parenchymal nodule must be dealt with as malignant until proved otherwise, since extirpation of the smallest possible malignancy gives the best prognosis. The authors also regard the persistent infiltrative density with equal suspicion and stress the importance of studying such radiographic changes with careful attention to comparison of present findings with all available previous radiographs.

A more selective approach is recommended in mass screening. Individual patients, particularly those over 40, should be educated to have radiographs made once or twice a year, by the same installation, to facilitate comparisons of present and past radiographs. It is urged that all roentgenograms be kept available for future comparison, since subtle changes are best seen by comparative studies.

III. *Heart and Circulation*

"CARDIAC EFFECTS OF ISOPROTERENOL, NOREPINEPHRINE AND EPINEPHRINE IN COMPLETE A-V HEART BLOCK DURING EXERIMENTAL ACIDOSIS AND HYPERKALEMIA." By SANTIAGO V. GUZMAN, M.D., ANTONIO C. DELEON, JR., M.D., JAMES W. WEST, Ph.D., and SAMUEL BELLET, M.D., Philadelphia General Hospital and University of Pennsylvania School of Medicine. *Circulation Research,* Volume 7, pages 666-672, July 1959. (T.I.R.C. grantee: Bellet)

Purpose of study: A number of sympathomimetic amines have been effective in restoring cardiac activity during Stokes-Adams attacks and cardiac arrest from other causes, but they fail to restore cardiac beating or to increase the slowed ventricle rate in some of these states. Since molar sodium lactate produced favorable response when these amines were ineffective, studies were undertaken in an attempt to elucidate some of the underlying mechanisms in the alteration of cardiac rhythm and response to sympathomimetic amines at various pH levels.

Procedure: Complete heart block was produced in mongrel dogs and respiratory acidosis was induced by inhalation of 20 percent CO_2 in oxygen for periods of 8 to 12 minutes. Metabolic acidosis was produced by intravenous infusion of ammonium chloride for a period of 3½ to 4 hours. Hyperpotassemia was produced by infusion of isotonic potassium chloride over a period of 30 minutes to one hour. Serial arterial blood samples were obtained at appropriate periods in each of the experimental conditions: acidosis and hyperkalemia, for determining changes in pH, CO_2, O_2, K, Na and Cl.

In another series of experiments the circumflex branch of the left coronary artery was catheterized in order to administer drugs directly to a localized portion of the heart in doses large enough to elicit local response without producing systemic effects.

Findings: Ammonium chloride acidosis resulted in decreases in pH and total blood CO_2 content associated with a significant rise in potassium and a fall in serum sodium levels, concomitant with a slowing of the cardiac ventricle rate and electrocardiographic changes similar to the findings in hyperpotassemia. Partial correction of the acidosis by intravenous infusion of molar sodium lactate resulted in a return to normal and in some instances to an increase in idioventricular rate significantly greater than the control rate.

Before and after acidosis, the response of each animal to various intravenous doses of the different sympathomimetic amines was determined, and a nearly linear correlation was found between the decrease in response to the amines and the degree of acidosis. In addition, there was a diminished rise in blood pressure. The intracoronary injections before acidosis resulted in increases in idioventricular rate comparable to those following intravenous injections of these drugs. During acidosis, the positive chronotropic effect from intracoronary injections of these amines diminished according to the decrease in pH; injections of small amounts of molar sodium lactate or sodium bicarbonate at this time produced a transient change in pH only within the vicinity of the idioventricular pacemaker, but injections of the alkalizing agents were ineffective in increasing the cardiac ventricle rate in the absence of acidosis.

These studies therefore suggest that the refractoriness to the sympathomimetic amines is due to the lowered pH and that correction of the latter restores their effectiveness.

Other grantor: American Heart Association

"FUNCTIONAL CAPILLARY BEDS IN THE BEATING, KCl-ARRESTED AND KCl-ARRESTED-PERFUSED MYOCARDIUM OF THE DOG." By S. R. M. REYNOLDS, Ph.D., D.Sc., and M. KIRSCH, M.D.,

Department of Anatomy, University of Illinois College of Medicine, Chicago; and R. J. BING, M.D., Washington University School of Medicine, St. Louis, Mo. *Circulation Research,* Vol. 6, pages 600-611, September 1958.

"EFFECT OF INTERRUPTION OF CORONARY CIRCULATION ON METABOLISM OF THE ARRESTED HEART." By G. MICHAL, A. BEUREN, C. S. HOGANCAMP, and R. J. BING, Washington University School of Medicine. *American Journal of Physiology,* Vol. 195, pages 417-423, November 1958. (T.I.R.C. grantee: Bing*)

Purpose of studies: The first report deals with a morphologic study of the functional capillary vascular bed of hearts obtained under the following three conditions: (1) the heart still beating, (2) the heart arrested for varying periods of time, and (3) the heart arrested for varying periods of time and then reperfused. The second investigation concerns the influence of myocardial ischemia on certain functions of energy production of heart muscle.

Procedures: In both studies, the arrested heart was perfused *in situ* without interrupting coronary circulation, immediately after introduction of potassium chloride to provoke cardiac arrest. Special catheters were introduced permitting the collection of simultaneous blood samples from the inflow tubing and the coronary sinus. The samples were analyzed for oxygen, glucose, pyruvate, lactate, and ketone bodies upon perfusion, while perfusion was interrupted for varying periods, and also when resumed.

In part two of the first study excised organs were examined histologically. The blood contained in all blood vessels at the time of perfusion was trapped by simultaneous clamping of all vascular channels. Hearts reperfused after ½ to 5 hours' circulatory arrest were also examined by this technique. Erythrocyte studies permitted contrasting analyses of arteriolar, capillary and venule relationship upon perfusion after circulatory arrest, and in hearts which had remained *in situ* for the same lengths of time without reperfusion.

In part two of the second study, the chest of the animal was opened, and circulation stopped by severing the aorta. A sample of heart muscle was removed immediately from the left ventricle tube used in manometric experiments. The chest wound was covered for periods ranging from ½ to 4 hours and another sample of heart muscle taken. Thus, each animal served as its own control. Slices from the heart muscles were taken, at thicknesses within the limits for sufficient diffusion of oxygen, and transferred into Warburg vessels, gassed with 95% O_2 and 5% CO_2 for 10 minutes; readings of metabolic activity were started and continued at 10-minute intervals for 80 minutes.

Findings: In normal hearts, erythrocyte counts of open capillaries showed a uniform distribution in the right myocardium, and a gradient decreasing from the epicardium toward the endocardium in the left myocardium. Potassium-chloride arrested hearts showed a reverse gradient in the left myocardium. The erythrocytes were oriented in capillaries with their flat sides lying against the nearest myocardial cell in normal hearts, but few or none had this orientation in arrested hearts. Erythrocytes in the arrested hearts were swollen, rounded, or packed in clumps.

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The arrested hearts exhibited a weakening of the connective tissue elements of the myocardium. The weakening increased as the duration of myocardial arrest increased.

In the second study, experiments on the whole heart resulted in a gradual decline in myocardial oxygen usage after two hours. After four hours, oxygen usage was negligible. Severe alterations in carbohydrate metabolism were observed, such as a significant increase in myocardial lactate production. In experiments with tissue slices, myocardial oxygen usage declined more rapidly and aerobic glycolysis took place in the heart muscle.

Other grantors: Public Health Service, The Life Insurance Medical Research Fund, American Heart Association, and Burroughs, Wellcome and Co., Inc.

"THE EFFECT OF COMPLETE ISCHEMIA ON THE INTRACELLULAR ELECTRICAL ACTIVITY OF THE WHOLE MAMMALIAN HEART." By MILTON KARDESCH, M.D., Charles E. HOGANCAMP, M.D., and RICHARD J. BING, M.D., Department of Medicine, Washington University, and the Washington University Medical Service, Veterans Administration Hospital, St. Louis, Mo. *Circulation Research,* Vol. 6, pages 715-720, November 1958.

"TRANSMEMBRANE ELECTRICAL POTENTIALS IN VENTRICAL TACHYCARDIA AND FIBRILLATION." By CHARLES E. HOGANCAMP, M.D., MILTON KARDESCH, M.D., WILLIAM H. DANFORTH, M.D., and RICHARD J. BING, M.D. *American Heart Journal,* Vol. 57, pages 214-222, February 1959. (T.I.R.C. grantee: Bing)

Purpose of studies: Recent studies in this laboratory have shown that the contractile proteins prepared from human hearts retain their contractility as late as 12 hours after death of the patient, and that the oxidative enzymes in heart muscle remain fully active for several hours after interruption of coronary circulation. The first study is concerned with the effects of complete ischemia on the spontaneous ventricular action and resting potentials of the isolated whole dog's and rabbit's heart. The second study applies the micropuncture technique of Lung & Gerard (J. Cell. & Comp. Physiol. 34:383, 1949) to the study of individual myocardial fibers and attempts to relate electrical and mechanical activity in alternation of the heart in guinea-pigs.

Procedure: Seventeen rabbits and four small dogs were anesthetized and whole hearts were quickly removed from the animals and stripped of their pericardium. Perfusions begun less than 90 seconds after interruption of the circulation resulted in immediate forceful muscular contractions. The perfusions were maintained at a constant pressure, with the hearts kept in a bathing solution. Spontaneous left ventricular membrane action and resting potentials were obtained by microelectrodes and recorded. Following a period of stabilization, perfusion was suddenly stopped and the bathing solution replaced by another rendered oxygen free by prior saturation and continuous bubbling with nitrogen, thus producing anoxia.

In the second study hearts were quickly removed from anesthetized guinea pigs and bathed in a solution constantly gassed by a mixture of 95% oxygen and 5% CO_2. Transmembrane resting and action potentials

were recorded by microelectrodes. Electrical potentials were recorded from multiple superficial ventricular cells in each preparation. A sudden drop in potential evidenced entry into a fiber. After preliminary observations and recordings of contractions in the spontaneously beating or electrically driven ventricles, arrhythmias were produced by applying aconitine to the heart surface.

Findings: In the first study, spontaneous membrane action potentials persisted for 20 minutes following the cessation of coronary perfusion. The earliest change in the action potential was a shortening of its duration, followed by a progressive fall in the amplitude of the spike. The resting potential showed a diminution to 65% of the control value. Sudden exposure of the whole heart to anoxia markedly shortened the duration of the action potential four minutes after interruption of the coronary circulation.

The findings suggest that it is the active sodium pump mechanism that is injured by anoxia. It is possible that the changes observed in the membrane resting potential are due to the inhibition of oxidative phosphorylation by anoxia, which is very similar to the effects of metabolic inhibitors on electrical potentials in the heart.

In the second study, simultaneous recording of electrical activity from two fibers on the ventricular surface of the guinea pig heart revealed partial synchronism of action potentials when the fibers were in close proximity, but not when the explored fibers were at a distance. Single fiber electrical alternans was frequently observed during ventricular tachycardia and was associated with mechanical alternans. It would appear that alternation of the heart, both electrical and mechanical, may be related to alternate variations in behavior of individual fiber membrane and contractile elements.

Other grantors: American Heart Association, Public Health Service, The Life Insurance Medical Research Fund, Burroughs, Wellcome & Co., Inc., and the Veterans Administration.

"THE HEART IN ANOXIA AND ISCHEMIA." By WILLIAM H. DANFORTH and RICHARD J. BING, Department of Medicine, Washington University, St. Louis, Mo. *British Journal of Anesthesia,* Vol. 30, pages 456-465, October 1958.

"THE SURVIVAL OF EXCITABILITY, ENERGY PRODUCTION AND ENERGY UTILIZATION IN THE HEART." By MILTON KARDESCH, M.D., CHARLES E. HOGANCAMP, M.D., and RICHARD J. BING, M.D. *Circulation,* Vol. 18, pages 935-945, November, 1958.

"MYOCARDIAL METABOLISM." By R. J. BING, J. D. CHOUDHURY, G. MICHAL, and K. KAKO. *Annals of Internal Medicine,* Vol. 49, pages 1201-1215, November 1958.

"MYOCARDIAL EFFICIENCY." By R. J. BING and G. MICHAL. *Annals of the New York Academy of Sciences,* Vol. 72, Article 12, pages 555-558, February 6, 1959 (T.I.R.C. grantee: Bing)

All four studies present reviews of work by the authors and others. Principal conclusions in the first are that the completely anoxic heart will continue to function as a pump only so long as the resting membrane potential remains sufficiently high and action potentials are generated and propagated. The action potentials decline and disappear over about 20

minutes of complete ischemia. At this time, neither the metabolic processes nor the contractile proteins are irreversibly damaged.

The second review discusses the difference in survival time of the three major biological functions of the anoxic heart muscle. Cardiac excitability is most vulnerable, while energy production and particularly energy utilization survive longer. As the ability of any organ to function depends upon the resistance of the weakest link, the loss of excitability of cardiac muscle which is induced rapidly by ischemia leads to death of the whole organism.

The third discussion is a review of cardiac metabolism of the beating and the arrested heart, a matter of increasing importance to the heart surgeon and the internist. It also deals with disturbances of cardiac metabolism in congestive heart failure and in myocardial ischemia and anoxia. In congestive heart failure in man, metabolic changes are absent and myocardial oxygen consumption is normal. In spontaneous heart failure, myocardial oxygen consumption and efficiency are diminished, but the oxygen extraction ratio remains unchanged. In experimental myocardial infarction in animals and in hemorrhagic shock, on the other hand, the heart muscle releases lactate and pyruvate into the coronary vein blood. The same observation was made after interruption of the coronary circulation for varying periods of time.

To date, coronary artery catheterization has failed in the detection of metabolic alterations in heart muscle cells which would either precede cell death or take place in cells in which metabolic changes are reversible. Future studies probably will be directed toward a combination of metabolic balance studies utilizing coronary sinus catheterization and enzymatic studies on heart muscle slices. This can best be accomplished by the use of radioisotopes.

The fourth review finds that the decline in cardiac efficiency in a number of clinical conditions, particularly congestive heart failure, results from lowered myocardial energy production rather than energy utilization.

"CHANGES IN TRANSMURAL CENTRAL VENOUS PRESSURE IN MAN DURING HYPERVENTILATION." By JOHN W. ECKSTEIN and WILLIAM K. HAMILTON, Hemodynamic Laboratory, Cardiovascular Research Laboratories, Department of Internal Medicine, and Division of Anesthesiology, Department of Surgery, State University of Iowa College of Medicine, Iowa City. *Journal of Clinical Investigation,* Vol. 37, pages 1537-1541, November 1958. (T.I.R.C. grantee: Eckstein)

Purpose of study: If the demonstrated shift of large amounts of blood from the forearm veins during hyperventilation was generalized in the periphery of the body, the volume of blood in the central venous reservoir might increase; but the fall in peripheral venous pressure which also occurs during hyperventilation implies that central venous pressure also decreases. This study was undertaken to assess the nature and magnitude of changes in mean *transmural* central venous pressure during hyperventilation.

Procedure: Simultaneous measurements of central venous pressure; intrapleural pressure (gauged by changes in esophageal pressure, which tend to be parallel); *transmural* central venous pressure (the difference between the first two measurements); and end-expiratory CO_2 concentration, were

made on a group of medical students in resting condition and again after at least two minutes of maximal inspiration and passive expirations. The hyperventilation was practiced while breathing air, while breathing 5% CO_2, and while breathing 5% CO_2 after intravenous administration of 5 mg. of phentolamine methanesulfonate.

Findings: Mean intrapleural pressure fell about the same amount in each kind of hyperventilation. Mean central venous pressure fell regularly during air hyperventilation, but remained essentially unchanged during CO_2 hyperventilation; it fell significantly, however, after phentolamine administration. The pressure distending the central veins (transmural pressure) increased during hyperventilation in almost all experiments because intrapleural pressure fell more than central venous pressure. Transmural pressure was significantly greater during CO_2 than during air hyperventilation because central venous pressure did not fall appreciably.

The increase in transmural central venous pressure supports the suggestion that blood shifts centrally during hyperventilation. The enhanced effect upon CO_2 addition is consistent with the suggestion that blood is pumped less rapidly from the central veins. This could be attributed to the slower heart rate with CO_2 hyperventilation. It could also mean that diastolic ventricular tone is increased with the higher concentration of CO_2 in the inspired gas. The response to phentolamine administration is additional evidence that CO_2 breathing is associated with an increase in the circulating level of catechol amines.

"CIGARETTE SMOKING, SERUM-CHOLESTEROL, BLOOD PRESSURE, AND BODY FATNESS OBSERVATIONS IN FINLAND." By MARTTI KARVONEN, M.D., Ph.D., and ESKO ORMA, M.D., Institute of Occupational Health, Helsinki, Finland; ANCEL KEYS, M.A., Ph.D., JOSEF BROZEK, Ph.D., and FLAMINIO FIDANZA, M.D., Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis. (Dr. Fidanza is now at the Institute of Human Psychology, University of Naples, Italy.) *The Lancet*, March 7, 1959, pages 492-494. (T.I.R.C. grantee: Keys)

Purpose of study: Both retrospective and prospective statistical studies show higher mortality rates from all causes and (in some papers) from ischemic heart disease among smokers than among non-smokers. In the absence of evidence that habitual cigarette smoking directly accelerates atherogenesis or increases susceptibility to thrombosis, argument as to whether a cause-and-effect relationship exists between smoking and heart disease is speculative. Therefore, it was pertinent to inquire whether smokers tend to differ from non-smokers in characteristics other than smoking habit, such as serum cholesterol levels, arterial blood pressure, and relative obesity.

Procedure: An epidemiological study was begun in 1956 of men living in rural areas of East and West Finland, later expanded to include men of the fire department and business and professional men in Helsinki. In both rural areas men aged 20-59 were selected in a number of small communities, excluding civil servants, temporary residents, and those known to be ill. Most of the men were farmers or timber workers, but car drivers, mechanics and shopkeepers were also sampled. The men were thought to be representative of their age groups in the two areas, but this was not

necessarily true of the Helsinki sample. All the men were asked to report for examination in the morning without breakfast, but no instructions were given about smoking. There were 360 regular cigarette smokers and 165 men who never smoked in the survey.

Findings: At all ages and in all regions (except in the 50-59 age group in West Finland) the smokers had significantly higher average serum cholesterol values than the non-smokers. At all ages and in all regions the smokers tended to have slightly lower blood pressures than the non-smokers; the difference in systolic blood pressure was highly significant, that in diastolic blood pressure significant but less impressive. In both rural areas the smokers tended to be slightly thinner than the non-smokers, but in Helsinki there was no clear relation between smoking and body fatness.

The data from Finland display interesting differences between smokers and non-smokers but they do not explain the origin of the differences, and the conclusion that habitual cigarette smoking causes serum cholesterol to rise, blood pressure to fall, and body to lose fat is not justified. The kind of person who smokes, in contrast to the one who is not inclined to take up smoking, may be simply so constituted otherwise that he tends to have elevated serum cholesterol, low blood pressure, and to be relatively thin.

Other grantors: American Heart Association, Finnish Heart Association, the Science Board of the State of Finland, and the Sigrid Juselius Stifelse (Helsinki).

"FURTHER STUDIES IN CHOLESTEROL LEVELS IN THE JOHNS HOPKINS MEDICAL STUDENTS: THE EFFECT OF STRESS AT EXAMINATIONS." By CAROLINE BEDELL THOMAS, M.D., and EDMOND A. MURPHY, M.D., Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Md., *Journal of Chronic Diseases,* Vol. 8, pages 661-668, December 1958. (T.I.R.C. grantee: Thomas)

Purpose of study: As part of a long term study on possible precursors of hypertension and coronary disease, continuous studies have been made of the cholesterol levels of successive classes of medical students. The class of 1961, entering in the fall of 1957, was the fourteenth class registered in the study. It was decided to obtain cholesterol determinations on that class during the final anatomy examination to compare with the levels obtained soon after admission, during a period of stress in which a human cadaver was being dissected by the students for the first time, and during a normal study period. The final anatomy examination is probably the greatest stress encountered by the students as a group during their four academic years.

Procedure: Body weight, blood pressure and heart rate were measured, then blood was drawn for cholesterol determination and a circulating eosinophil count: on admission to medical school (Test I — October 1957), during final anatomy examinations (Test II — Jan. 6-10, 1958), and during laboratory study periods (Test III) between Jan. 21 and April 24, 1958. The statistical analysis is based on the protocols of the 52 men with complete cholesterol data for all three tests. Women were excluded because of fluctuations in cholesterol levels with the menstrual cycle.

Findings: The mean cholesterol levels at Tests I and II were significantly higher than at Test III, a finding consistent with the hypothesis that serum cholesterol levels rise during periods of stress. The eosinophil data support

the view that the students were experiencing more stress at Test II than at Test III. There were no significant differences in body weight between the three tests and no pronounced variations in habits of exercise, diet, or smoking.

Other grantor: National Heart Institute.

"OBSERVATIONS ON SOME POSSIBLE PRECURSORS OF ESSENTIAL HYPERTENSION AND CORONARY ARTERY DISEASE. VI. COMPARISON OF THE CIRCULATORY REACTIVITY TO THE COLD PRESSOR TEST AND TO THE SMOKING TEST." By CAROLINE BEDELL THOMAS, M.D., FACP, and EDMOND A. MURPHY, M.D., Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Md. *Annals of Internal Medicine,* Vol. 50, pages 970-980, April 1959. (T.I.R.C. grantee: Thomas)

Purpose of study: It is generally recognized that hypertensive patients show greater lability of blood pressure under various forms of stress than do normotensive persons, and that such lability is a characteristic of the individual which *precedes* the development of hypertension. Normotensive subjects with exaggerated responses to the cold pressor and to the ballistocardiographic smoking tests have been called "normal hyperreactors;" as there are marked individual differences in reaction to both tests, a closer definition of normality appears desirable.

Procedure: Cold pressor tests, with blood pressure and pulse rate measured after immersing the hand in ice water, and ballistocardiograph tests after smoking one cigarette, were conducted on 386 healthy medical students, including 30 women, about a year apart. Of the men, 162 were smokers, 155 non-smokers, 19 former smokers, and the habits of 20 were unknown.

Findings: In the cold pressor test, the average blood pressure rises for men and women were quite similar. This was not true of the heart rate change, where the men showed a mean rise of 5.4 beats per minute while the women showed a much smaller change, plus 1.2 beats per minute. There was a tendency for the magnitude of response to decrease with age. No significant differences in response were found among the different smoking categories.

In the ballistocardiographic smoking test, the mean rises of systolic and diastolic pressure were smaller than those found in the cold pressor test. Average blood pressure changes were not significantly different in women and men, but the heart rate change of the female subjects was significantly greater than that found among the men. Here also there was a tendency for the responses to decrease with age. Non-smokers showed a significantly greater rise in systolic pressure than smokers and a smaller rise in heart rate than either smokers or former smokers.

It is interesting that the significant correlation between systolic response to smoking and diastolic response to cold was *positive* in men and *negative* in women.

Each of the three measurements in the two tests showed approximately a normal distribution, so that they all appear to be continuous variables. Any dividing line or cutting point to distinguish hyperreactors is therefore purely arbitrary. It is concluded that independent information is gained

from each test. Accordingly, both tests may contribute to the appraisal of individual circulatory reactivity, and one test does not replace the other.

Other grantors: National Heart Institute and Veterans Administration. "VARIABILITY OF CHOLESTEROL LEVELS IN INDIVIDUAL JOHNS HOPKINS MEDICAL STUDENTS, WITH OBSERVATIONS ON STOPPING SMOKING, VITAMIN B_{12} ADMINISTRATION AND ACUTE INFECTION." By CAROLINE BEDELL THOMAS and FRIEDA FAIMAN EISENBERG, Department of Medicine, The Johns Hopkins University School of Medicine. *Bulletin of the Johns Hopkins Hospital*, Volume 105, pages 14-50, July 1959.

Purpose of study: Previous studies of cholesterol levels in medical students showed that although the mean level tended to rise with age for the group, the factor of age alone did not account for the wide differences in cholesterol level, nor did age always parallel this level in a given subject on repeated tests. All the varied findings suggest that there is much more to be learned about the biological variables affecting the cholesterol level in a given individual, and it was decided to study the effect of three such variables on the cholesterol level in a few individuals in a particular environment, rather than the more usual cross-sectional study of a large population.

Procedures: Five male and one female students who volunteered to stop smoking during the tests and five male controls filled out detailed questionnaires on smoking habits; an appraisal of fat content of the diet also was made, and serum cholesterol levels were determined before, during and following the tests. During period I both SS and C groups smoked the customary amount; during period II the SS students gave up smoking and the C group continued to smoke, as they did during period III; in the latter, two SS students resumed smoking after 7-8 weeks of not smoking, one after the tenth week, and the others withdrew from the experiment after 5-7 weeks. During period IV vitamin B_{12} was administered to the 5 C subjects and to one SS subject who had resumed smoking. Serum cholesterol levels were also studied in seven of the subjects who had acute respiratory infections.

Findings: The six "stop smoking" volunteers, who started with low cholesterol values (an interesting degree of self-selection as compared to their classmates), showed no definite tendency to develop significantly lower cholesterol values during the experiment; only one showed a marked gain in weight, beginning soon after stopping smoking. Two of the six subjects showed a significant fall in mean cholesterol levels during vitamin B_{12} administration; there were no consistent changes in body weight, blood pressure or heart rate during this test. Acute infections of the influenzal type were accompanied by a sharp transitory fall in cholesterol levels in three subjects.

When subjects were grouped by the grand mean of all cholesterol values per man, the total variability and the biological variability of those with higher values were on the whole greater than of those with lower values. At any given grand mean cholesterol level, there were individual differences in the tendency toward constancy of cholesterol level, on the one hand, or toward fluctuation, on the other.

Other grantor: National Heart Institute.

"EFFECT OF NICOTINE ON CHOLESTEROL-INDUCED ATHEROSCLEROSIS IN THE RABBIT." By DUANE G. WENZEL, Ph.D., JAMES A. TURNER, M.D., and DONALD KISSIL, M.S., Department of Pharmacology, University of Kansas School of Pharmacy, and Veterans Administration Hospital, Kansas City, Mo. *Circulation Research,* Vol. 7, pages 256-261, March 1959. (T.I.R.C. grantee: Wenzel)

Purpose of study: It has been reported that nicotine increases the plasma cholesterol levels of male rabbits on a cholesterol-fortified diet. The purpose of this study was to determine the effect of graded doses of nicotine plus a high cholesterol diet on atherosclerosis, using the following criteria: serum cholesterol and phospholipid levels, electrocardiographic changes before and after ergonovine stress, and gross and micropathology of the heart and contiguous vessels.

Procedure: Six groups, of twelve albino New Zealand six-week old female rabbits each, were fed various experimental diets, and determinations of body weight, serum cholesterol, and phospholipid and electrocardiographic activity (both with and without ergonovine) were made initially and every eight weeks thereafter for 24 weeks. All surviving animals were then killed. In addition to examination for microscopic pathology, the degree of aortic sclerosis was grossly graded on a 0 to 4 scale (less than 2% up to 80% of the total surface involved).

Findings: The addition to the cholesterol regimen of nicotine, equivalent in amount to human consumption of two packs of cigarettes daily, did not significantly affect body weight, serum cholesterol or lipid phosphorus, or gross aortic atherosclerosis under the conditions of this test. The nicotine-cholesterol groups demonstrated greater mortality as well as greater electrocardiographic and pathologic evidence of cardiac involvement and peripheral vascular changes than did the cholesterol-only or nicotine-only rabbits. It is suggested that prolonged constriction of the vessels involved or other undefined actions of nicotine created the proper physical and/or metabolic environment for the deposition of cholesterol. The observation that the small coronary vessels were thickened and fibrotic in the nicotine only group would strengthen this possibility.

IV. *Tobacco Chemistry*

"METABOLISM OF METHIONINE AND PECTIN ESTERIFICATION IN A PLANT TISSUE." By CLIFFORD S. SATO, RICHARD U. BYERRUM, PETER ALBERSHEIM, and JAMES BONNER, Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena. *Journal of Biological Chemistry,* Vol. 233, pages 128-131, July 1958. (T.I.R.C. grantee: Bonner)

Purpose of study: Although the transfer of methyl from methionine to nitrogen is known to occur in the synthesis of plant alkaloids, the mechanism of such methylation and of methyl transfer in general is little understood. In this study, the transfer of the methyl of methionine to pectin, the principal methyl acceptor of plant tissues, has been investigated, as well as possible intermediates in the transfer. In addition, the genesis of methyl

groups from formaldehyde and the role of phosphoryl choline in methyl transfer has been studied.

Procedure: The experiments have been carried out with oat seedling coleoptiles excised from the plant and floated in the desired quantity of metabolite. It has been shown in earlier studies that the transfer of methyl to pectin methyl ester groups is speeded in this tissue by the presence of the plant hormone indoleacetic acid. The effect of indoleacetic acid on methyl genesis and transfer has therefore also been investigated.

Findings: Oat sections oxidize methionine to its sulfoxide, both *in vivo* in living and in homogenized sections. The reaction is essentially non-reversible. Methionine sulfoxide can donate its methyl group to pectin and to protopectin as well as transfer its methyl group to methionine with the formation of methyl methionine. Methionine sulfoxide cannot, however, serve as a methyl acceptor.

"METABOLISM OF NICOTINE TO (+)-GAMMA-(3-PYRIDYL)-GAMMA-METHYLAMINOBUTYRIC ACID." By HERBERT MCKENNIS, JR., LENNOX B. TURNBULL, and EDWARD R. BOWMAN, Department of Pharmacology, Medical College of Virginia, Richmond, *Journal of the American Chemical Society*, Vol. 80, pages 6597-6600, Dec. 20, 1958. (T.I.R.C. grantee: Dr. P. S. Larson)

Purpose of study: In a previous communication from this laboratory, data were presented to show that gamma-(3-pyridyl)-gamma-methylaminobutyric acid arises during the metabolism of nicotine in the dog, authenticating in part a general hypothetical route for such metabolism. Further study of the chemical reactions and substances formed was necessary to a fuller understanding of the metabolic process.

Procedures: Following intravenous administration of (—) nicotine (I), the urine of dogs was acidified to pH 2 and then placed on a column which retained all Koenig-positive material; this material was removed by elution with N/1 ammonia water and separated into six zones upon paper chromatography with ammonia-ethanol-butanol. The zones, absent from control urine, were compared for position by cochromatography with nicotine and a number of its derivatives; the material at Rf 0.15 was gamma-3-pyridyl-gamma-methylaminobutyric acid (II). Nicotine, cotinine and unknown at Rf 0.61 were extracted and the component at Rf 0.15 retained (9.2% of the administered dose of nicotine). On heating, this material became chloroform soluble and changed in Rf value to that of cotinine, leading to unequivocal chemical identification.

Findings: Gamma-(3-pyridyl)-gamma-methylaminobutyric acid, in the metabolism of nicotine, is on formal grounds both a precursor and metabolite of cotinine. It was observed frequently that older samples of urine contained increasingly large amounts of material in the cotinine zone of chromatograms and increasingly smaller concentrations of II. This spontaneous lactamization is in agreement with the previously noted instability of the compound. Irrespective of the initial mode of its formation, some II will serve as a precursor of cotinine in the biological systems studied.

It is further concluded that any major direct conversion of cotinine to II would depend upon the presence of an enzymatic system. Experiments in which cotinine is supplied to the animal instead of nicotine may serve to

elucidate the possible role of cotinine as an intermediate in the metabolism of nicotine.

"A CONSTANT-RATE INFUSION APPARATUS." By QUENTIN S. MCKENNIS, EDWARD R. BOWMAN, and HERBERT MCKENNIS, JR., Department of Pharmacology, Medical College of Virginia, Richmond. *Toxicology and Applied Pharmacology,* Vol. 1, pages 61-64, January 1959. (T.I.R.C. grantee: Dr. P. S. Larson)

In the course of studies in which dogs were given aqueous solutions of nicotine intravenously over an 8-hour period, it was desirable to develop a moderately priced apparatus for simultaneous infusion at constant rate to six or more animals. The wide range of applicability of the simple device developed for this purpose prompted a report on it.

"THE BACTERIAL OXIDATION OF NICOTINE. I. NICOTINE OXIDATION BY CELL-FREE PREPARATIONS. II. THE ISOLATION OF THE FIRST OXIDATIVE PRODUCT AND ITS IDENTIFICATION AS (1)-6-HYDROXYNICOTINE." By L. I. HOCHSTEIN and SYDNEY C. RITTENBERG, Department of Bacteriology, University of Southern California, Los Angeles. *Journal of Biological Chemistry,* Vol. 234, pages 151-155 and 156-160, January 1959. (T.I.R.C. grantee: Rittenberg)

Purpose of study: Various pathways have been postulated for nicotine degradation involving an initial attack at either the pyridine or the pyrrolidine rings. In an effort to avoid the difficulties inherent in the use of complex systems, studies of nicotine metabolism were attempted at the enzyme level employing crude and fractionated extracts derived from a bacterium. Identification of the first metabolite isolated continued the study.

Procedure: A gram-negative rod isolated from soil and capable of using nicotine as its sole source of carbon was grown in a medium containing trace salts, nicotine, yeast extract, and other compounds. The inoculated medium was treated with sterile air at room temperature, and the culture was harvested after it reached the maximum stationary phase. Ultraviolet absorption spectra of cell-free extracts were determined, and oxygen consumption and carbon dioxide production were measured.

Findings: Nicotine oxidation proceeded through a series of sharp changes of rate occuring after the uptake of 0.5, or multiples of 0.5, μmole of oxygen per μmole of nicotine. Each point of change of rate coincided with the temporary accumulation of intermediates in the oxidation sequence, and none of the serially accumulated intermediates are oxidized until their precursors are exhausted. The first oxidative product of nicotine degradation by the soil bacterium was identified as (1)-6-hydroxynicotine by comparison with a synthesized compound.

"DARK FIXATION OF CO_2 BY SUCCULENT LEAVES: CONSERVATION OF THE DARK FIXED CO_2 UNDER DIURNAL CONDITIONS." By GEORGE KUNITAKE and PAUL SALTMAN, Department of Biochemistry and Nutrition, School of Medicine, University of Southern California, Los Angeles. *Plant Physiology,* Vol. 33, pages 400-403, November 1958.

"DARK FIXATION OF CO_2 BY TOBACCO LEAVES." By GEORGE KUNITAKE, CLYDE STITT, and PAUL SALTMAN. *Plant Physiology,* Vol. 34, pages 123-127, March 1959. (T.I.R.C. grantee: Saltman)

Purpose of studies: Although earlier studies strongly suggest that dark fixation of CO_2 by succulents and non-succulents is ubiquitous, the nature of the biochemical reactions involved is not fully understood. Leaves of the succulent *Bryophyllum calycinum* Salisb. and of the non-succulent *Nicotinia tabacum* were studied concurrently.

Procedure: Random samples of the leaves of the foregoing plants were placed respectively in an apparatus which permits exposure to $C^{14}O_2$ in total darkness for varying periods, and identification of the labeled photosynthetic and dark products was made on aliquots of 80% ethanol soluble fractions.

Findings: Tobacco leaves have the ability to incorporate $C^{14}O_2$ in the dark into several organic and amino acids, identified by paper chromatography and radioautography. The initial rate of dark fixation in tobacco is more rapid than that of the succulent *B. calycinum*, but the total amount of $C^{14}O_2$ fixed by tobacco after extended periods is much less than by the succulent leaves. No carbohydrates or phosphorylated sugars are labeled after extended periods of dark exposure, suggesting that light is needed to furnish the specific power to reverse glycolytic processes.

"QUANTITATIVE STUDIES OF SCOPOLETIN IN CIGARETTE SMOKE AND TOBACCO." By CHAO-HWA YANG, YASUSHI NAKAGAWA, and SIMON WENDER, Chemistry Department, University of Oklahoma, Norman. *Analytical Chemistry*, Vol. 31, pages 2041-2044, December 1958. (T.I.R.C. grantee: Wender)

Purpose of study: Discovery that the mainstream smoke from cigarettes commonly used in the United States contained scopoletin has necessitated the development of procedures to determine quantitatively the amount of this compound in the smoke and in the cigarette tobacco before smoking.

Procedure: Cigarettes of 24 different brands and sizes were analyzed individually or in composite by smoking on a standard smoking apparatus, condensing the smoke in flasks, and by grinding the tobacco from cigarettes with the filters and paper removed and extraction on a steam bath. Determination of the scopoletin content was made by paper chromatography.

Findings: The amounts of scopoletin in micrograms per gram of tobacco ranged from 66 to 123 for regular, menthol, filter, and king size cigarettes, and from 41 to 48 for denicotinized cigarettes. The amounts of scopoletin recovered from smoke, in terms of micrograms per gram of cigarette smoked ranged from 14.0 to 27.4 for regular, menthol and king size cigarettes, from 12.1 to 17.9 for filter cigarettes, and from 10.3 to 11.4 for denicotinized cigarettes. A significant percentage of scopoletin passes through the filters of the filter cigarettes analyzed.

Other grantor: Atomic Energy Commission

V. Other Studies

"MEDICAL USES OF TOBACCO, PAST AND PRESENT." By H. SILVETTE, Ph.D., P. S. LARSON, Ph.D., and H. B. HAAG, M.D., Department of Pharmacology, Medical College of Virginia, Richmond. *Virginia Medical Monthly*, Vol. 85, pages 472-484, September 1958. (T.I.R.C. grantee: Haag)

In this review of the literature, no attempt has been made to re-cover the earliest history of tobacco as a drug. The year 1828 has been taken as the dividing line between the "ancient" and "modern" pharmacological history of tobacco, because nicotine was discovered by Posselt & Reimann in that year. Although tobacco is no longer an *official* drug, it still is frequently used as a folk-medicine, the authors report. A bibliography of 166 papers on the subject is appended.

"Tobacco made its world-wide conquests under guise of a drug," an epilogue to the study says. "But the dangers attendant upon its therapeutic use, as well as the discovery of safer, more efficacious, and often more specific remedies for many of the diseases in which tobacco was once employed, have taken *Herba Panacea* out of the pharmacopoeias, and, indeed (in spite of more scientific therapeutic tests by some modern physicians), generally out of medical practice as well. Tobacco as a drug is now primarily of only medico-historical interest.

"Or is it? Have we not forgotten that most essential and characteristic of all the pharmacological — and hence therapeutic — properties of nicotine: its unique biphasic action on the higher, if not the highest nervous centers of man? Have we not forgotten that, by virtue of this effect, tobacco has been used and celebrated for over three centuries as what we are now pleased to term a 'tranquilizing' drug — a 'tranquilizer,' moreover, with perhaps a higher therapeutic index of safety and a lower incidence of dangerous side-reactions than any white tablet or colorful capsule similarly chronically used."

"STUDIES IN TOBACCO HYPERSENSITIVITY. III. REACTIONS TO SKIN TESTS AND PERIPHERAL VASCULAR RESPONSES." By VINCENT J. FONTANA, M.D., WALTER REDISCH, M.D., ROSE LEE NEMIR, M.D., MARJORIE K. SMITH, M.D., KURT DECRINIS, M.D., and MARION B. SULZBERGER, M.D., New York University Post-Graduate Medical School and New York University Research Service, Goldwater Memorial Hospital, New York University-Bellevue Medical Center, New York City. *Journal of Allergy,* Volume 30, pages 241-249, May-June 1959. (T.I.R.C. grantee: Sulzberger)

Purpose of study: The divergent views and findings in the literature on tobacco sensitivity prompted the further investigation of the possibility that certain tobacco effects are based on a specific allergic susceptibility of particular persons rather than on obligatorily toxic products in tobacco smoke.

Procedures: Skin reactions to tobacco extracts were tested on 641 healthy adults and 294 children and historical data on the subjects were collected. Tobacco extracts were prepared from Burley, Virginia and Turkish cured tobaccos and from a blend, in equal parts, of the tobacco and paper from six principal brands of cigarettes; two types of the mixed tobacco extracts were tested: one was defatted with toluene before aqueous extraction, removing some organic substances which might act as allergens; the other was not treated before extraction.

In a second experiment, 80 healthy adults who had been skin tested with tobacco extract were studied before smoking a cigarette and at varied intervals thereafter, and a series of cardiovascular measurements were obtained.

Findings: Among the adults, 18.4% of the smokers showed markedly

positive reactions to one or more of the tobacco extracts, compared to 13.8% of the non-smokers; the difference was not found to be statistically significant. There were more "marked" reactions to the mixed tobacco extract that was not defatted (9.8%) than to the defatted extract (6.9%). Sex and age did not seem to play any role in the incidence of the reactions to tobacco in the various groups tested.

Peripheral vascular symptoms among 377 smokers tested with Virginia tobacco extract were about twice as common (26 to 30% compared with 11 to 15%) in smokers with positive skin tests than in smokers with negative skin reactions. In 260 non-smokers, the incidence of these symptoms was about the same (10 to 19% compared with 11 to 14%, respectively) whether the skin-test reaction to tobacco was positive or negative.

In the group of smokers with a personal history of allergic manifestations, 53.5% were found to react positively to one or more of the tobacco extracts. In contrast, only 18.7% of the smokers without history of allergy reacted to tobacco extract. Among non-smokers, the figures were 40.9% and 14.7% respectively for those with and without personal allergic histories. The skin sensitivity to tobacco could well be more readily acquired by the generally allergic person than by the generally less allergic one on intimate or casual contact with tobacco smoke and products.

No significant differences were found in electrocardiographic, ballistocardiographic, and blood flow measurement changes after smoking. On the other hand, skin temperature changes were noted in 28% of the smokers giving positive skin-test reactions to tobacco and in only four per cent of those giving negative reactions. The difference is statistically significant.

Of the 294 children aged one to five years skin-tested, 11.5% reacted positively to one or more of the tobacco extracts. Questioning of the parents of 269 of the children revealed no evidence of allergic manifestations; of these, six per cent reacted positively to tobacco extract. Of the 25 children found to be allergic, 64% have evidence of skin sensitivity to tobacco. Practically all the children were exposed to one or more smokers within the immediate family at home. There were no age or sex differences.

On the basis of these findings, there is a suggestion that the skin test with tobacco may be helpful as a "screening" test to aid in determining the possible importance of tobacco as an etiological factor in certain forms of peripheral vascular symptomatology.

Following are titles of abstracts that appeared in the 1957 and 1958 Reports of the Scientific Director:

"CARDIAC EFFECTS OF INTRACORONARY ARTERIAL INJECTIONS OF NICOTINE." By JAMES W. WEST, B.S., SANTIAGO V. GUZMAN, M.D. and SAMUEL BELLET, M.D., Department of Pharmacology, University of Pennsylvania School of Medicine and Division of Cardiology, Philadelphia General Hospital, Philadelphia, Pa. *Circulation Research*, Vol. 6, No. 3, pages 389-395, May 1958. (T.R.C. grantee: Bellet)

"CONTRACTILITY AND EXTRACTABILITY OF HEART ACTOMYOSIN AFTER DEATH." By L. DETTLI, M.D., and R. J. BING, M.D., Department of Experimental Medicine, the Medical College of Alabama, Birmingham. *Circulation Research*, Vol. 14, No. 5, pages 519-522, September 1956.

"CONTRACTILE PROPERTIES OF ACTOMYOSIN THREADS AND BANDS PREPARED FROM DOGS' HEARTS." By L. DETTLI, M.D., and R. J. BING, M.D. *American Journal of Physiology*, Vol. 187, No. 1, pages 145-150, October 1956.

"EFFECT OF CIGARETTE SMOKING ON CORONARY BLOOD FLOW AND MYOCARDIAL METABOLISM." By L. M. BARGERON, JR., M.D., D. EHMKE, M.D., F. GONLUBOL, M.D., A. CASTELLANOS, M.D., A. SIEGEL, M.D., and R. J. BING, M.D. *Circulation*, Vol. 15, No. 2, pages 251-257, February 1957.

"DIAGNOSTIC VALUE OF ACTIVITY OF MALIC DEHYDROGENASE AND PHOSPHOHEXOSE ISOMERASE. Preliminary Report of Findings in Patients with Myocardial Infarction and Liver Disease." By RICHARD J. BING, M.D., ALBERTO CASTELLANOS, M.D., and ABRAHAM SIEGEL, M.S., *Journal of the American Medical Association*, June 8, 1957, pages 647-650.

"DETERMINATIONS OF DPN (TPN) THIAMINE, AMINO ACIDS AND AMINO SUGARS IN BLOOD OF ANIMALS WITH EXPERIMENTAL MYOCARDIAL INFARCTION." By G. CABRERA, M.D., A. BEUREN, M.D., and R. J. BING, M.D., Department of Medicine, Washington University School of Medicine, St. Louis, Mo. *Circulation Research*, Vol. 6, No. 1, pages 15-19, January 1958.

"METABOLIC STUDIES ON THE ARRESTED AND FIBRILLATING PERFUSED HEART." By ALOIS BEUREN, M.D., CHARLES SPARKS, M.D., and RICHARD J. BING, M.D., FACC. *American Journal of Cardiology*, Vol. 1, No. 1, pages 103-112, January 1958.

"CONTRACTILITY OF ACTOMYOSIN BANDS PREPARED FROM NORMAL AND FAILING HUMAN HEARTS." By K. KAKO and R. J. BING*. *Journal of Clinical Investigation*, Vol. 37, No. 3, pages 465-470, March 1958. (T.I.R.C. grantee: Bing)

"A SURVEY OF COMPOUNDS FOR ACTIVITY IN THE SUPPRESSION OF MOUSE SEBACEOUS GLANDS." By FRED G. BOCK and RHODA MUND, Roswell Park Memorial Institute, Buffalo, N. Y. *Cancer Research*, Vol. 18, No. 8, pages 887-892, September 1958.

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Hospital, Bronx, N. Y., and Clinical Professor of Surgery, Albert Einstein College of Medicine, New York City; MYRON SALTZ, M.D., HAROLD W. HARROWER, M.D., Veterans Administration Hospital, Providence, R. I., and Boston University of School of Medicine, Boston, Mass.; and DOROTHY H. BURKE, A.B., Veterans Adminnstration Hospital, Providence, R. I., *Gastroenterology*, Vol. 33, No. 6, pages 959-967, December 1957.

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Recipients of Grants

Following is a list of all recipients of grants approved by the Scientific Advisory Board since initial grants were made in late 1954. It should be noted that some of the projects have been completed.

GRANTEE AND INSTITUTION	PROJECT TITLE *("C" indicates project completed; "P" indicates a report on work has been published)*
CLARENCE M. AGRESS, M.D., *Chief, Cardiovascular Laboratory,* Veterans Administration Center, Los Angeles 25, California	The Measurement of the Functional Status of the Human Heart by Frequency Spectrum Analysis of its Vibrational Energy
D. MURRAY ANGEVINE, M.D., *Professor of Pathology,* University of Wisconsin Medical School, Madison, Wisconsin	Pathologic — Anatomic Study of Cellular Changes in Human Bronchi (C)
FREDERICK W. BARNES, JR., M.D., PH.D., *Associate Professor of Medicine and Physiological Chemistry,* Johns Hopkins University School of Medicine, Baltimore 5, Maryland	The Role of Hyperplasia in Tissue Response to Chronic Damage (C—P)
SAMUEL BELLET, M.D., *Director, Division of Cardiology,* Philadelphia General Hospital, Philadelphia, Pennsylvania	The Effect of Nicotine on Cardiac Irritability in the Presence of Reserpine, and the Effect of Nicotine on Coronary Blood Flow of Dogs with Coronary Insufficiency (P)
RICHARD J. BING, M.D., *Professor of Medicine and Chairman, Department of Medicine,* Wayne State University College of Medicine, Detroit, Michigan	The Effect of Smoking on the Coronary Blood Flow and Certain Phases of Myocardial Metabolism in Patients with Arteriosclerotic or Hypertensive Cardiovascular Disease (P) Studies in Cellular Physiology of Heart Muscle Measurement of Coronary Blood Flow by Means of Radioactivated Albumin
FRED G. BOCK, M.S., PH.D., *Senior Cancer Research Scientist,* Health Research Inc., Roswell Park Division, Buffalo, New York (see Moore)	Investigation of the Biological Effects of Cigarette Smoke (C—P)
JAMES F. BONNER, PH.D., *Professor of Biology,* California Institute of Technology, Pasadena, California	Enzymatic Study of Methylation Reactions in Plant Tissue (C—P)
TOM G. BOWERY, PH.D., *Pesticide Residue Laboratory, Chemistry Department,* North Carolina State College, Raleigh, North Carolina	TDE and Endrin Residues in Cigarette Smoke (P)

GRANTEE AND INSTITUTION	PROJECT TITLE
JOSEF M. BROZEK, PH.D., *Professor, Laboratory of Physiological Hygiene,* University of Minnesota School of Public Health, Minneapolis, Minnesota (now *Professor and Chairman,* Department of Psychology, Lehigh University, Bethlehem, Pennsylvania)	Biological Characteristics of Men and Their Smoking Habits (C—P)
E. M. BUTT, M.D., *Professor of Pathology,* University of Southern California School of Medicine; *Chief Pathologist,* Los Angeles County Hospital, Los Angeles 33, California	Study of Trace Metal Storage of Pulmonary and Liver Tissue by Spectographic and Chemical Methods (C) Pathologic — Anatomic Study of Cellular Changes in Human Bronchi (C)
RICHARD U. BYERRUM, PH.D., *Professor of Chemistry,* Michigan State University, East Lansing, Michigan	Biosynthesis of the Pyridine Ring of Nicotine
WILLIAM H. CARNES, M.D., *Professor of Pathology,* University of Utah College of Medicine, Salt Lake City, Utah	Pathologic — Anatomic Study of Cellular Changes in Human Bronchi (C)
LEOPOLD CERECEDO, PH.D., *Professor of Biochemistry,* Fordham University, New York 58, N. Y. (now *Professor of Biochemistry and Nutrition,* University of Puerto Rico, San Juan, Puerto Rico)	A Study of Early Chemical Changes in the Lungs of Tumor-Bearing Rats and Mice (C—P)
HANS T. CLARKE, D.Sc., *Professor of Biochemistry,* Columbia University College of Physicians and Surgeons, New York, N. Y. (see Gottschall)	Proteolytic Activities of the White Blood Cells of Men and the Effect of White Blood Cell Activities of Carcinogens, Nutrition and Other Influences (C)
JULIUS H. COMROE JR., M.D., *Director, Cardiovascular Research Institute,* University of California Medical School, San Francisco 22, California	The Effect of Smoking Upon Airway Resistance
DEAN M. CONNORS, M.D., *Associate Director, Department of Laboratory Medicine,* St. Mary's Hospital, Madison 5, Wisconsin	A Study of the Alterations in the Human Bronchial Wall Occurring with Ageing, with Particular Emphasis on Elastic Tissue Changes and Associated Changes in the Bronchial Lumen Size
PHILIP COOPER, M.D., *Chief, Surgical Service,* Veterans Administration Hospital, Bronx, New York; *Clinical Professor of Surgery,* Albert Einstein Medical College, Bronx	A Study of the Effects of Cigarette Smoking on Levels of Gastric Acid, Pepsin and Uropepsin (C—P) A Study of the Effect of Extracts of Tobacco on Cultures of Tumor and Normal Cells, Animal Transplants of Tumor Tissue from Tissue Cultures
R. F. DAWSON, PH.D., *Professor of Botany,* Columbia University, New York, N.Y.	An Investigation of the Metabolism of Pyridine Compounds in the Tobacco Plant
EDWARD F. DOMINO, M.D., *Assistant Professor of Pharmacology,* University of Michigan, Ann Arbor, Michigan	Effects of Tobacco Smoke and Nicotine on the Central Nervous System

GRANTEE AND INSTITUTION	PROJECT TITLE
JOHN W. ECKSTEIN, M.D., *Assistant Professor of Internal Medicine,* College of Medicine, State University of Iowa, Iowa City, Iowa	Responses of the Peripheral Veins in Man to the Intravenous Administration of Nicotine (P) Foot Blood Flow Responses to Smoking in the Presence of Hyperlipemia and Hypertension
HANS L. FALK, PH.D., *Assistant Professor of Biochemistry,* University of Southern California School of Medicine, Los Angeles 33, California	Examination of Cigarette Paper and Cigarette Smoke Condensates for Aromatic Polycyclic Hydrocarbons (C) A Compilation of Fluoresence Spectra of Polycyclic Aromatic Hydrocarbons and Closely Related Compounds Which Are of Interest in the Study of Air Pollutants, and Cigarette Smoke in Relation to Lung Cancer Etiology
DANA L. FARNSWORTH, M.D., *Henry K. Oliver Professor of Hygiene and Director of University Health Services,* Harvard University, Cambridge 38, Massachusetts (see Heath and McArthur)	Personality and Smoking in College Graduates: A Fifteen-Year Follow-up Study (C)
FRANK C. FERGUSON, JR., M.D., *Chairman, Department of Pharmacology,* Albany Medical College, Albany 8, New York	Effects of Tobacco Smoke Upon the Function of the Cardiovascular System in Animals and Man
RUSSELL S. FISHER, M.D., *Chief Medical Examiner,* State of Maryland; *Professor of Legal Medicine,* University of Maryland Medical School, Baltimore, Maryland	Pathologic — Anatomic Study of Cellular Changes in Human Bronchi (C)
B. L. FREEDLANDER, M.D., *Director of Cancer Research,* Mt. Zion Hospital, San Francisco 15, California (see French)	Experiments on the Carcinogenic and Cocarcinogenic Action of Tobacco Products (P)
FREDERICK A. FRENCH, *Research Associate,* Mt. Zion Hospital and Medical Center, San Francisco, California (see Freedlander)	Carcinogenicity, Cocarcinogenicity and Anti-carcinogenicity of Dietary Factors in Relation to Pulmonary Tumors. Possible Interrelationship of Tobacco Bases and Dietary Factors. Chemical Studies on Pyridine Bases Including Niacin Analogs
JACK FREUND, M.D., *Lecturer in Pharmacology, Assistant in Medicine,* Medical College of Virginia, Richmond 19, Virginia	Correlation of Multitechnical Procedures Performed on the Peripheral Circulation of Normal Individuals in Recumbent and Erect Positions and After Exercise Before and After Sham and Actual Smoking A Study of the Effects of Cigarette Smoking on the Peripheral Circulation of Individuals with Arteriosclerosis Obliterans and other Peripheral Vascular Diseases, Utilizing Multi-technical Procedures

GRANTEE AND INSTITUTION	PROJECT TITLE
GEORGE O. GEY, M.D., *Director, Finney-Howell Cancer Research Laboratory, Department of Surgery,* Johns Hopkins Hospital, Baltimore, Maryland	Fellowships for Studying the Culture of Human Lung Tissue and the Effects of Known and Possible Carcinogenic Agents Upon Such Tissue
GERTRUDE Y. GOTTSCHALL, PH.D., *Assistant Professor of Biochemistry, Department of Pathology and Microbiology,* The Rockefeller Intsitute for Medical Research, New York 21, N.Y. (see Clarke)	Proteolytic Activities of the White Blood Cells of Man and the Effect on White Blood Cell Activities of Carcinogens, Nutrition and Other Influences
A. CLARK GRIFFIN, PH.D., *Head of Biochemistry Department,* M.D. Anderson Hospital and Tumor Institute, University of Texas Medical Center, Houston 25, Texas	The Effect of Exposure to Cigarette Smoke on the Induction of Cancer by Chemical Compounds (C—P)
MORTON I. GROSSMAN, PH.D., M.D., *Associate Clinical Professor of Medicine,* University of California Medical Center, Los Angeles 24, California	The Effect of Smoking on Certain Gastric Functions
CARL C. GRUHZIT, PH.D., M.D., *Associate in Physiology and Pharmacology,* University of Pennsylvania Graduate School of Medicine, Philadelphia 4, Pennsylvania (now *Lecturer in Physiology,* University of Hong Kong)	Pharmacologic Study of Nicotine and Related Alkaloids (C)
H. B. HAAG, M.D., *Professor of Pharmacology,* Medical College of Virginia, Richmond 19, Virginia	Preparation for Publication of a Book on the Biologic Aspects of Tobacco and its Smoke (P)
JOSEPH H. HAFKENSCHIEL, M.D., *Director, Cardiopulmonary Unit,* Lankenau Hospital, Philadelphia 31, Pennsylvania	Measurement of Coronary Blood Flow, Cardiac Work and Cardiac Oxygen and Carbohydrate Metabolism in Normotensive Subjects Before and After Intravenous Nicotine and After Smoking Standard Cigarettes (C—P)
RICHARD J. HAVEL, M.D., *Assistant Professor of Medicine,* University of California Medical School, San Francisco, California	A Study of the Effects of Smoking and Nicotine Administraton on Sympathoadrenal Function and Fatty Acid Metabolism
HERBERT R. HAWTHORNE, M.D., *Chairman, Department of Surgery,* University of Pennsylvania Graduate School of Medicine, Philadelphia 4, Pennsylvania	Attempts to Induce Pulmonary Neoplasms in Experimental Animals by Exposure of the Tracheo-Bronchial System to Tobacco Smoke (C)
CLARK W. HEATH, M.D., *Physician, Department of Hygiene,* Harvard University, Cambridge 38, Massachusetts (see Farnsworth and McArthur)	Personality and Smoking in College Graduates: A Fifteen-Year Follow-Up Study (C—P)

GRANTEE AND INSTITUTION	PROJECT TITLE
PAULINE HEIZER, PH.D., *Research Associate in Cytology and Cytochemistry*, San Francisco Institute of Medical Sciences, San Francisco 15, California (see Richards)	A Comparative Study of Early Histological and DNA Changes in the Epidermis of Two Strains of Mice (C57 Blacks and Swiss Websters) After Daily Applications of Whole Cigarette Smoke Condensate (Alone and Combined with Croton Oil) and the Carcinogens 20-Methylcholanthrene and 3,4-Benzpyrene
RUSSELL L. HOLMAN, M.D., *Professor and Head, Department of Pathology*, Louisiana State University School of Medicine, New Orleans 12, Louisiana	Pathologic — Anatomic Study of Cellular Changes in Human Bronchi (C)
	The Influence of Tobacco Smoking on Acute Myocardial Infarction
F. HOMBURGER, M.D., *President*, Bio-Research Laboratories, Inc., Cambridge, Massachusetts	Studies on Carcinogenesis and the Bioassay of Carcinogenic Agents (P)
	Comparative Studies of Effects of Various Tobacco Smoke Condensates on Skins of Mice
JERRY HART JACOBSON, M.D., *Director of Electrophysiology*, New York Eye and Ear Infirmary, New York 3, N. Y.	A Comparison of Electroretinography as a Means of Evaluating the Effect of Vasoconstrictor Drugs Upon Cerebral and Retinal Circulation With Other Techniques for This Determination (C)
ANDREW A. KANDUTSCH, PH.D., *Staff Scientist*, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine	To Determine to What Extent Tobacco Tar Mimics the Action of Carcinogenic Hydrocarbons in the Skin and Other Tissues
ANCEL KEYS, PH.D., *Professor of Physiological Hygiene and Director, Laboratory of Physiological Hygiene*, University of Minnesota School of Public Health, Minneapolis 4, Minnesota	Characteristics of Men, Including Smoking, in Populations Differing in the Incidence of Coronary Heart Disease (C—P)
JOSEPH B. KIRSNER, M.D., *Professor of Medicine*, University of Chicago School of Medicine, Chicago, Illinois	The Effect of Tobacco Smoking Upon Basal Gastric Secretions in Man
KENNETH P. KNUDTSON, M.D., *Assistant Chief, Laboratory Service*, Veterans Administration Hospital, Seattle, Washington; *Professor of Pathology*, University of Washington Medical School, Seattle, Washington	Pathologic-Anatomic Study of Cellular Changes in Human Bronchi (C)
ALVIN I. KOSAK, PH.D., *Associate Professor of Chemistry*, Washington Square College, New York University, New York 16, N. Y.	The Isolation and Identification of Certain Lower-Boiling Components of Cigarette Smoke (C)
MARVIN KUSCHNER, M.D., *Professor of Pathology*, New York University College of Medicine; *Director of Pathology*, Bellevue Hospital, New York 16, N. Y.	Pathologic-Anatomic Study of Cellular Changes in Human Bronchi (C)
THOMAS C. LAIPPLY, M.D., *Associate Professor of Pathology*, Northwestern University Medical School, Chicago, Illinois	Pathologic-Anatomic Study of Cellular Changes in Human Bronchi (C)

GRANTEE AND INSTITUTION	PROJECT TITLE
PAUL S. LARSON, PH.D., *Professor of Pharmacology,* Medical College of Virginia, Richmond 19, Virginia	Enzymatic Transformations of Nicotine and Related Compounds (P)
CECILIE LEUCHTENBERGER, PH.D., *Senior Biologist and Cytochemist,* Children's Cancer Research Foundation, Boston, Massachusetts	A Correlated Histological, Cytological and Cytochemical Study of the Tracheo-Bronchial Tree from Mice Exposed to Cigarette Smoke (P)
ROBERT H. LINNELL, PH.D., *Associate Professor of Chemistry,* University of Vermont, Burlington, Vermont	The Oxidation of Nicotine by Gaseous Oxygen: Mechanism, Products and Kinetics
DAVID E. MANN, JR., PH.D., *Associate Professor of Pharmacology,* Temple University School of Pharmacy, Philadelphia 40, Pennsylvania	Effect of Tobacco Smoke and Tobacco Residues on Methylcholanthrene-Induced Skin Carcinogenesis in Mice (C)
CHARLES C. McARTHUR, PH.D., *Psychologist to the University Health Services,* Harvard University, Cambridge 38, Massachusetts (see Farnsworth and Heath)	Social and Personal Determinants of Smoking Behavior (C—P)
CHARLES B. McCANTS, PH.D., *Associate Professor of Soils,* School of Agriculture, North Carolina State College, Raleigh, North Carolina	Arsenic Content of Soils and Absorption by the Tobacco Plant
KELLY T. McKEE, M.D., *Associate Professor of Medicine,* Medical College of South Carolina, Charleston, South Carolina	Study of Lung Function in Smokers and Non-Smokers (C—P)
JAMES G. MILLER, M.D., PH.D., *Professor of Psychiatry and Psychology, and Director,* Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan	The Behavioral Effects of Smoking Under Stress
HUGH MONTGOMERY, M.D., *Associate Professor of Medicine,* University of Pennsylvania Medical School, Philadelphia 4, Pennsylvania	Influence of Tobacco Smoking on the Blood Flow of Skin and of Muscles of Extremities in Sympathectomized and Unsympathectomized Subjects (C) Influence of Nicotine (i.v.) and Tobacco Smoking on Blood Flow in Human Skin and Skeletal Muscle
GEORGE E. MOORE, PH.D., M.D., *Director,* Roswell Park Memorial Institute, Buffalo 3, New York (see Bock)	An Investigation of the Physiological Effects of Direct Inhalation of Tobacco Smoke by Laboratory Animals and the Study of the Biological Response of Laboratory Animals to Continuous Ingestion of Diet-Tobacco Product Mixtures (C)
HURLEY LEE MOTLEY, M.D., *Professor of Medicine and Director, Cardio-Respiratory Laboratory,* University of Southern California School of Medicine, Los Angeles 17, California	A Study of the Effects of Smoking on Pulmonary Function (C—P)

GRANTEE AND INSTITUTION	PROJECT TITLE
WILLIAM S. MURRAY, Sc.D, *Research Associate and Administration Director,* Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine	The Production of Genetically Controlled Animals and Tumors for Use in Experimental Research on Tobacco in Relation to Health by (a) the Expansion of Known Inbred Stocks and Sources of Tumor Supply; (b) the Production of Such Hybrids or Heterozygous Types as Become Necessary; and (c) the Relation of this Material to Specific Experimental Work at the Laboratory (C)
DONALD M. PACE, PH.D., *Professor of Physiology and Director, Institute for Cellular Research,* University of Nebraska, Lincoln, Nebraska	Fellowships for Training in Tissue Culture Techniques Study of the Effects of Tobacco Smoke Constituents on Various Strains of Tissue Cells Cultivated *In Vitro*
EDWARD W. PELIKAN, M.D., *Associate Professor of Pharmacology and Experimental Therapeutics,* Boston University School of Medicine, Boston, Massachusetts	A Study of Structure-Activity Relationships Among Drugs Which Affect Nicotine-Sensitive Physiological Mechanisms
C. M. POMERAT, PH.D., *Professor of Cytology,* University of Texas Medical Branch, Galveston, Texas	Fellowships for Studying the Culture of Human Lung Tissue and the Effects of Known and Possible Carcinogenic Agents Upon Such Tissue
H. R. PRATT-THOMAS, M.D., *Professor of Pathology,* Medical College of South Carolina, Charleston, South Carolina	Application of a New Bio-Assay Technique in Examination of Cigarette Smoke Condensates for Possible Carcinogens (C—P) Pathologic-Anatomic Study of Cellular Changes in Human Bronchi (C)
VICTOR RICHARDS, M.D., *Professor of Surgery, Executive Head, Department of Surgery,* Stanford University School of Medicine, San Francisco 15, California (see Heizer)	A Comparative Study of the Effects of Whole and Fractional Extracts of Cigarette Smoke and Those of Known Carcinogens on (1) The Cytology and Nuclear DNA Content of Epidermis in Various Strains of Mice and/or (2) The Cytology and Nuclear DNA Content of Lung and Epithelium of the Bronchial Tree of Mice and Hamsters (C)
R. H. RIGDON, M.D., *Professor of Pathology and Director, Laboratory of Experimental Pathology,* University of Texas Medical Branch, Galveston, Texas	Effects of Tobacco Smoke Condensate on the Respiratory Tract and Other Tissues of the Duck (P)
SYDNEY C. RITTENBERG, PH.D., *Professor of Bacteriology,* University of Southern California, Los Angeles 7, California	The Bacterial Degradation of Nicotine and Related Compounds. The Objective of the Project is the Elucidation of the Intermediary Metabolism of Nicotine Oxidation (P)
BENJAMIN A. RUBIN, PH.D., *Assistant Professor of Public Health and Preventive Medicine,* Baylor University College of Medicine, Houston, Texas	An Evaluation of the Phenomenon of Tumor Growth Enhancement as an Assay for Carcinogens Among the Polycyclic Hydrocarbons and Related Compounds

GRANTEE AND INSTITUTION	PROJECT TITLE
WILLIAM O. RUSSELL, M.D., *Pathologist-in-chief,* M.D. Anderson Hospital and Tumor Institute, University of Texas Medical Center, Houston 25, Texas	Pathologic-Anatomic Study of Cellular Changes in Human Bronchi (C)
PAUL D. SALTMAN, PH.D., *Assistant Professor of Biochemistry,* University of Southern California School of Medicine, Los Angeles 7, California	The Enzymatic Mechanism for the Dark Fixation of CO_2 by Tobacco (C—P) Some Aspects of Amino Acid Metabolism in Tobacco Leaves
ALVIN R. SCHMIDT, PH.D., *Director of Counseling,* Tufts University, Medford, Massachusetts	Study of Attitudes Toward, as Well as Extent, Type and History of, Tobacco Smoking in a Young College Population. Study of Some of the Relationships of Smoking and Nonsmoking to Family History, Type of Secondary Schooling, Academic Interests, Academic Achievement, and Social Relationships Characteristics
ISAAC SCHOUR, D.D.S., PH.D., D.Sc., *Professor of Histology and Embryology and Dean,* University of Illinois College of Dentistry, Chicago 12, Illinois	Histologic Changes in the Oral, Pharyngeal and Nasal Tissues of Experimental Animals Subjected to Tobacco Smoke (C—P)
MAURICE S. SEGAL, M.D., *Clinical Professor of Medicine,* Tufts University School of Medicine, Boston, Massachusetts; *Director, Department of Inhalation Therapy,* Boston City Hospital, Boston, Massachusetts	Effects of Cigarette Smoking on Lung Function in Normal Subjects and Patients With Certain Respiratory Disease Conditions (C—P) Relationship of Cigarette Smoking to Chronic (Obstructive) Pulmonary Emphysema
CARL C. SELTZER, PH.D., *Research Fellow in Physical Anthropology,* Harvard University, Cambridge, Massachusetts	Morphology and Smoking in College Graduates: A Fifteen-Year Follow-Up Study
CHARLES E. SHERWOOD, M.D., *Assistant Professor of Radiology,* University of Rochester School of Medicine and Dentistry, Rochester, New York	Investigation Into the Natural History of Carcinoma of the Lung With Particular Reference to the Radiographic Appearance of Such Processes, the Earliest Manifestation of Cancer on Chest X-Ray Photographs and the Tabulation of the Relationship of Smoking Habits and Occupation With the Incidence of Lung Cancer (C—P)
DAVID L. SIMON, M.D., *Instructor in Medicine and Fellow in Cardiovascular Research,* University of Cincinnati College of Medicine and Cardiac Laboratory, Cincinnati General Hospital, Cincinnati 29, Ohio	The Effects of Chewing Tobacco on the Cardiovascular System of Man (C—P) The Effects of Pipe Smoking and Cigar Smoking on the Cardiovascular System of Man
T. M. SONNEBORN, PH.D., *Distinguished Service Professor of Zoology,* Indiana University, Bloomington, Indiana	Checking and Extending the Stephano *Paramecium* Test for Carcinogenicity

GRANTEE AND INSTITUTION	PROJECT TITLE
SAM SOROF, PH.D., *Research Associate*, The Institute for Cancer Research and Lankenau Hospital Research Institute, Philadelphia 11, Pennsylvania	Chemical and Physical Studies of the Tissue Proteins Involved in Chemical Carcinogenesis (C—P)
FREDERICK J. STARE, PH.D., *Professor of Nutrition*, Harvard School of Public Health, Boston 15, Massachusetts	Experimental Studies of Cancer Utilizing a New Technique to See if Various 'Tars' Extracted From Tobacco May Incite the Formation of Lung Tumors (C)
MARION B. SULZBERGER, M.D., *Professor and Chairman, Department of Dermatology and Syphilology*, New York University-Bellevue Medical Center, New York 16, New York	Investigation of the Effects of Tobacco on the Human Vascular System, Based on the Fact that Certain Tobacco Effects are Due to Allergic Susceptibility of Specific Individuals Rather than to Obligatorily Toxic Products in Tobacco Smoke, and that Patients with Occlusive Vascular Diseases Respond Differently than Healthy Smokers (P)
CAROLINE BEDELL THOMAS, M.D., *Associate Professor of Medicine*, Johns Hopkins University School of Medicine, Baltimore 5, Maryland	The Significance of Different Individual Patterns of Circulatory Response to Cigarette Smoking (P) Studies of Genetic Differences Between Smokers and Nonsmokers (P). Studies of Psychological Differences Between Smokers and Nonsmokers as Shown by Comparison of Figure Drawings
TISSUE CULTURE ASSOCIATION (*President*, Dr. Margaret R. Murray, *Associate Professor of Anatomy*, Columbia University College of Physicians and Surgeons, New York, N.Y.)	To Assist in Establishment of Summer Training Course in Tissue Culture Techniques at University of Colorado Medical School, Denver, Colorado (C)
JANET TRAVELL, M.D., *Associate Professor of Clinical Pharmacology*, Cornell University Medical College, New York 21, New York	Effects of Nicotine in the Rabbit with Experimental Coronary Atherosclerosis
E. D. WARNER, M.D., *Professor of Pathology*, State University of Iowa College of Medicine, Iowa City, Iowa	Pathologic-Anatomic Study of Cellular Changes in Human Bronchi (C) Correlation of Bronchial Epithelial Changes with Comparable Changes in Other Organs—A Pathologic-Anatomic Study
RICHARD L. WECHSLER, M.D., *Clinical Physiologist*, Montefiore Hospital Institute of Research, Pittsburgh 13, Pennsylvania	Effect of Cigarette Smoking on Cerebral Blood Flow, Cerebral Metabolism, Blood Gases, Blood pH, Arterial Pulse Pressure Curves, Electrocardiograms, and Electroencephalograms (C)
RUSSELL W. WELLER, M.D., *Pathologist*, Memorial Hospital of Chester County, West Chester, Pennsylvania; *Associate Professor of Pathology*, Hahnemann Medical College, Philadelphia 2, Pennsylvania	Pathologic-Anatomic Study of Cellular Changes in Human Bronchi A Selected, Extended and Detailed Study of Human Bronchial Mucosa

GRANTEE AND INSTITUTION	PROJECT TITLE
SIMON H. WENDER, PH.D., *Research Professor of Chemistry,* University of Oklahoma Research Institute, Norman, Oklahoma	A Qualitative and Quantitative Study of the Individual Polyphenol Content of Cigarette Tobacco and of the Smoke and 'Tars' Resulting from Cigarette Smoking, and Also Study of the Fate of These Compounds in the Animal Respiratory System (C—P)
DUANE G. WENZEL, PH.D., *Professor of Pharmacology,* School of Pharmacy, University of Kansas, Lawrence, Kansas	The Determination of the Chronic Effects of Orally Administered Nicotine on Serum Cholesterol and Phospholipids; the Electrocardiographic Response to Ergonovine; and the Vascular Pathology of Cholesterol-fed Rabbits (C—P)
FREDERICK E. WHISKIN, M.D., C.M., *Director, Division of Health and Personality Equilibrium,* The Age Center of New England, Inc., Boston 16, Massachusetts	Pilot Study of the Smoking Habits of Age Center Members
J. EDWIN WOOD, M.D., *Professor of Medicine,* Medical College of Georgia, Augusta, Georgia	The Effect of Prolonged Inhalation of Tobacco Smoke and of Prolonged Abstinence from the Use of Tobacco on the Peripheral Vascular Response to Acute Inhalation of Tobacco Smoke in Man (C—P)
JOHN P. WYATT, M.D., *Professor of Pathology,* St. Louis University School of Medicine, St. Louis 4, Missouri	An Investigation Into the Nature of the Pigmentary Lesion in Centrilobular Emphysema